ARCHIVES OF PATHOLOGY

VOLUME 48 DECEMBER 1949 NUMBER 6

BRONCHIAL CARCINOMA

A Practical Method of Early Diagnosis

K. R. CROSS, M.D.

T. E. CORCORAN, M.D.

T. J. COOPER, M.D.

AND

S. N. LANDIS, M.D.

DES MOINES, IOWA

IN THIS PAPER we outline and illustrate with cases our experience in diagnosing bronchogenic carcinoma by examination of bronchial aspirations and washings. This material can be embedded, sectioned and stained. As demonstrated in many illustrations dealing with "the cytologic diagnosis of cancer," and as stated by some, cell groups or islands are actually obtained. These, in our experience, amount to minute biopsy specimens and show less distortion in sections than is produced when smears are prepared. The same criteria of cancer, namely, anaplasia and loss of polarity, can be applied to these minute specimens as to larger ones obtained with the knife. The information contained in the published material dealing with the morphologic aspects of these cancers in smears can also be applied. This method of examination does not require a prohibitive amount of time.

Carcinoma of the lung is of great importance. In our experience, it has been the most frequent cancer encountered exclusive of skin cancer. The treatment is surgical removal, the technic of which has been improved remarkably in the recent past. The feature of successful management which now demands most attention is early diagnosis.

The great importance in suspected cases of cancer of examining exfoliated material from the bronchial tree, whether aspirated through the bronchoscope or collected as sputum, and whether examined in smears or in prepared sections, as emphasized by Hunter and Richardson,[1] has been amply demonstrated. By this method, neoplasms can often be diagnosed before other positive evidence can be elicited.

From the Department of Pathology, Veterans Administration Hospital.

Published with the permission of Chief Medical Director, Department of Medicine and Surgery, Veterans Administration, who assumes no responsibility for the opinions expressed or conclusions drawn by the authors.

1. Hunter, W. C., and Richardson, H. L.: Surg., Gynec. & Obst. **85**:275, 1947.

Lesions inaccessible to the bronchoscope can also be proved to exist when biopsy is impossible or the specimen inadequate, as pointed out by Gibbon, Clerf, Herbut and DeTuerk,[2] by Woolner and McDonald[3] and as illustrated in all 5 of the cases to be reported here.

This method so enhances the likelihood of the early diagnosis which contributes to successful treatment that its use can no longer be restricted to large centers and clinics. The general pathologists in the smaller hospitals and clinics, where most of these patients are first seen, can no longer ignore it.

Cancer of the lung has been diagnosed by examination of fluid from the respiratory tract for many years. The first to report such a case was Hampeln,[4] in 1887, who examined unstained smears of fresh sputum. In 1918 he[5] published a second paper concerning 25 cases of pulmonary cancer, in 13 of which carcinoma cells were found in the sputum. He stressed the diagnostic importance of isolated neoplastic cells. Betschardt,[6] using the technic of blocking and sectioning, studied fragments in sputum and reported a case of bronchogenic carcinoma diagnosed by this method in 1895. With this method other investigators (Sauerbruch,[7a] Fishberg,[7b] Weller,[7c] Sweany[7d] and Edwards[7e]) also have made positive diagnoses.

Stockard and Papanicolaou[8] started a study of the morphologic and cyclic variations of exfoliated cells obtained from the vaginas of guinea pigs. Papanicolaou[9] reported in 1928 that exfoliated cancer cells had been observed in human vaginal secretions. Little cognizance was taken of this finding until 1943, when Papanicolaou and Traut[10] published their monograph "The Diagnosis of Uterine Cancer by the Vaginal Smear."

Dudgeon and Wrigley[11] reported in 1935 that they had applied the wet film technic to the examination of sputum for the early diag-

---

2. Gibbon, J. H., Jr.; Clerf, L. H.; Herbut, P. A., and DeTuerk, J. J.: J. Thoracic Surg. **17**:419, 1948.

3. Woolner, L. B., and McDonald, J. R.: Surg., Gynec. & Obst. **88**:273, 1949.

4. Hampeln, P.: St. Petersburg Med. Wchnschr. **4**:137, 1887, cited by Woolner and McDonald.[3]

5. Hampeln, P.: Mitt. a. d. Grenzgeb. d. Med. u. Chir. **31**:672, 1918-1919.

6. Betschardt, E.: Virchows Arch. f. path. Anat. **142**:86, 1895.

7. (*a*) Sauerbruch, E. F.: J. A. M. A. **51**:808, 1908. (*b*) Fishberg, M.: Arch. Int. Med. **37**:745, 1926. (*c*) Weller, C. V.: Arch. Path. **7**:478, 1929. (*d*) Sweany, H. C.: Ann. Otol., Rhin. & Laryng. **43**:561, 1934. (*e*) Edwards, A. T.: J. Thoracic Surg. **4**:107, 1934.

8. Stockard, C. R., and Papanicolaou, G. N.: Am. J. Anat. **22**:225, 1917.

9. Papanicolaou, G. N.: Proc. Third Race Betterment, 1928, p. 528.

10. Papanicolaou, G. N., and Traut: The Diagnosis of Uterine Cancer by the Vaginal Smear, London, Commonwealth Fund, 1943, pp. 47.

11. Dudgeon, L. S., and Wrigley, C. H.: J. Laryng. & Otol. **50**:752, 1935.

nosis of bronchogenic carcinoma. Their method, devised by Dudgeon and Patrick [12] in 1927, consisted in making films of the suspected fluid, fixing these while wet in Schaudin's fluid and staining with hematoxylin and eosin. That method of diagnosis has gained in popularity and is now being used by numerous investigators, some of whom have varied the fixative or the stains. The examination of exfoliated cells has now been applied to the diagnosis of cancer of the lungs, uterus, kidneys, bladder, prostate and stomach.

The reports of the results obtained by using the wet film technic of Dudgeon and Patrick [12] or Papanicolaou,[13] with or without minor variations, reveal that the diagnosis can be made in nearly 75 per cent of proved cases of bronchial carcinoma. Only reports giving sufficient data as to the total number of patients with carcinoma who were examined and the results of those examinations are included here.

Dudgeon and Wrigley [11] found cancer cells in the sputum of 26 of 38 patients in whom the presence of bronchogenic carcinoma was proved or probable. Gowar [14] found cancer cells in the sputum of 36 of 65 patients with carcinoma of the lung. Papanicolaou [15] reported a positive diagnosis on the basis of examinations of sputum in 22 of 33 cases of proved or probable cancer of the lungs. Wandall,[16] examining sputum, found cancer cells in 84 of 100 cases of proved bronchial carcinoma. Farber and others,[17] examining both sputum and bronchial secretions, demonstrated cancer cells in 57 of 71 proved cases of bronchogenic carinoma. They found a close correlation of the results obtained when using both sputum and aspirated bronchial secretions. McKay, Ware, Atwood and Harken [18] found neoplastic cells in aspirated bronchial material in 40 of 54 cases of proved bronchogenic carcinoma. Herbut and Clerf [19] found neoplastic cells in aspirated bronchial material in 47 of 57 cases of proved carcinoma of the lung. In 25 of the last 27 cases their results were positive.

By profiting from the efforts of the earlier workers and with increasing experience and confidence, the authors of the more recent reports were able to reveal a greater degree of accuracy than was first thought possible with these methods.

It seems unnecessary to repeat descriptions of the specific cytologic features of neoplasms of the bronchial system. These have been pre-

12. Dudgeon, L. S., and Patrick, C. V.: Brit. J. Surg. **15**:250, 1927.
13. Papanicolaou, G. N.: Science **95**:438, 1942.
14. Gowar, F. J. S.: Brit. J. Surg. **30**:193, 1943.
15. Papanicolaou, G. N.: J. A. M. A. **131**:372, 1946.
16. Wandall, H. H.: Acta chir. Scandinav. (supp. 93) **91**:1, 1944.
17. Farber, S. M., and others: Dis. of Chest. **14**:633, 1948.
18. McKay, D. G.; Ware, P. F.; Atwood, D. A., and Harken, D. C.: Cancer **1**:208, 1948.
19. Herbut, P. A., and Clerf, L. H.: M. Clin. North America **30**:1384, 1946.

sented, first, in standard textbooks of pathology dealing with the microscopic appearance of these neoplasms, and second, in many excellent reviews of the cytologic diagnosis in which small aggregates and single cells are considered.[20] This is well covered by Woolner and McDonald,[8] and by Diggs,[21] whose illustrations are excellent. During the period covered in this report we have confined our diagnoses simply to suspicions of and the presence or the absence of cancer. Wandall [16] and others have gone further in typing these neoplasms on the basis of examinations of sputum or of bronchial secretions. We believe this is possible in the majority of cases, and the matter is under further study at this time.

It may be noted that in the examination of this material loss of polarity in cell groups and anaplasia of epithelial cells are the most important features to evaluate. Inasmuch as one may be dealing with a squamous cell carcinoma, an adenocarcinoma or a small, undifferentiated cell carcinoma, these features must be evaluated in each case. Variation from cell to cell in nuclear size, nuclear contour and density of the nuclear membrane—the last varying not only from cell to cell but from one zone to another in the same nucleus—and variation in the arrangement and density of the chromatin and in the size and position of the nucleoli thus become of the greatest importance. This variation from cell to cell becomes the key in this diagnosis and is much easier to evaluate when relatively undistorted cell groups are examined and adjacent cells can be compared. Wandall [16] in 1944 stressed these variations and concluded that rarely could the diagnosis of cancer be made by examining individual cells.

The term "cytologic diagnosis of cancer," assumes that the diagnosis is made by examination of individual cells. The majority of authors, however, in their discussions and particularly in illustrations, point to cell masses or groups. Several, including Wandall,[16] Papanicolaou,[15] and Dudgeon,[22] have pointed to the importance of cell groups. Liebow, Lindskog and Bloomer [23] stated, "Reliance was placed only upon groups of cells possessing an arrangement suggesting that of tissue, not merely upon individual atypical cells." Albers, McDonald and Thompson [24] considered cell clumps essential for the diagnosis of well differentiated carcinoma of the prostate on the basis of examination of prostatic secretions.

---

20. Herbut, P. A., and Clerf, L. H.: J. A. M. A. **130**:1006, 1946. Dudgeon and Wrigley.[11] Wandall.[16] Farber and others.[17]

21. Diggs, L. W.: Am. J. Clin. Path. **18**:293, 1948.

22. Dudgeon, L. S.: St. Thomas's Hosp. Rep. **1**:51, 1946.

23. Liebow, A. A.; Lindskog, G. E., and Bloomer, W. E.: Cancer **1**:223, 1948.

24. Albers, D. D.; McDonald, J. R., and Thompson, G. J.: J. A. M. A. **139**:299, 1949.

It has been emphasized by several authors that mitoses are rarely seen in smear preparations and that they are, therefore, not of great importance. In blocking and sectioning the material, we have observed them frequently in the cell groups and regard them of importance, just as in any other type of biopsy.

It is recommended that special attention be given to the reports of false positive diagnoses. Among these, squamous metaplasia, as emphasized by Wandall,[16] ranks high as a source of error. Some of the specific criteria of cancer must also be present.

Two recent cases in which we have made positive diagnoses, one of which was thought to represent our first false positive diagnosis, have gone on to present the clinical and roentgenographic pictures of cancer. In the first case the lesion was considered inoperable at the time of surgical intervention and a single biopsy revealed a nonspecific granulomatous lesion. In the second case a tuberculoma was removed and was thought to be the only lesion. These cases are not considered entirely proved or disproved and therefore are not included in our statistics. Although tubercle bacilli were not demonstrated in these cases, there may be represented here the coexistence of tuberculosis and neoplasms which Bergmann, Shatz and Flance [25] discussed and which was observed by us at autopsy in 2 recent cases.

Thus there have been no proved false positive diagnoses in our experience.

PROCEDURE

It is our practice in each case in which carcinoma of the lung is suspected to review the history, the physical findings and the roentgenograms. The patient is examined with the bronchoscope, and if lesions are seen, specimens are taken for biopsy, and in all cases aspirations and/or washings are obtained, regardless of other findings or procedures.

The material is immediately rinsed with solution of formaldehyde U. S. P. from the vial in which it was collected. It is then centrifuged at 1,000 to 2,000 revolutions per minute for ten to forty-five minutes. After a minimum of four hours' fixation, the supernatant fluid is carefully decanted, and the remaining button wrapped in filter paper if necessary. It is then dehydrated and embedded as any other surgical specimen. Sections are cut from the block and stained with hematoxylin and eosin. This procedure has been used in all cases herein reported.

Several features of this procedure should be emphasized. They have all been encountered, and their neglect has contributed much to the relatively poor results obtained in the early work. The section of tubing connecting the aspirator used with the vial should be washed in order to obtain the material therein. The material should not be centrifugated faster than 2,000 revolutions per minute or for more than forty-five minutes, to avoid laking of the cells. The material must be fixed promptly. The hematoxylin must be destained in water to a point at which nuclear details are distinct.

25. Bergmann, M.; Shatz, B. A., and Flance, I. J.: J. A. M. A. **138**:798, 1948.

We have recently adopted the policy of cutting four sections from four different levels because this will increase the likelihood of finding cancerous tissue, especially if the material has been centrifuged.

Hunter and Richardson [1] recommended that the material be fixed in a saturated solution of trinitrophenol (picric acid), and the cell mass separated by filtration. These cell masses may be easier to dehydrate and embed, presumably because of greater size and cohesiveness, but we have had insufficient experience with this method to evaluate it fully. Kraushaar and Bradbury [26] have reported the use of celloidin (pyroxylin) tips on centrifuge tubes which can be detached and embedded without disturbing the cell mass. Birge, McMullen and Davis [27] have recommended the use of powdered fibrinogen and thrombin to coagulate the sediment obtained by centrifugation.

Inasmuch as we examine all suspected patients with the bronchoscope, we have confined ourselves to the examination of aspirated material. In comparative studies, Herbut and Clerf [20] emphasized that bronchial secretions were superior to sputum as a source of carcinoma cells, and Woolner and McDonald [28] found the two materials equally satisfactory. Aspirated material is delivered as are other surgical specimens to the laboratory, and after centrifugation they are handled exactly as are other surgical specimens.

The professional staff's examination is conducted as with other surgical specimens, with discussion when indicated. Residents become acquainted with the material. Hunter and Fremont-Smith, in their discussion of the report of Pollard, Bryant, Block and Hall,[29] who diagnosed gastric neoplasms by cytologic examination of gastric secretions, pointed to the advantages of sections in those examinations, and Hunter stated, "It is my opinion that not until something better than the smear, namely, treating material as tissue, is adopted, will pathologists give any serious consideration to this method for the diagnosis of cancer."

The time and the care required of both technicians and pathologist have been great deterrents to the examination of smear preparations of sputum in the case of general pathologists. Farber and others [17] stated, "It is desirable that at least three to five slides be made from each of five daily specimens, and examined, before a report is given. At least 15 minutes per slide is required by our technicians. Suspicious slides require more time." If five slides are prepared each time and the recommended time spent on each examination, six hours and fifteen minutes per case would be consumed. That does not include the time required of the technician for preparation of the slides or that of the pathologist for final evaluation and diagnosis. As often recommended, the time required per patient may amount to seven or eight man-hours. This is prohibitive in the smaller hospital with a limited staff.

COMMENT

Several discussions dealing with the cytologic diagnosis of cancer, i. e., with the examination of stained smears of fluids which possibly contain exfoliated cancer cells, consider the appearance of individual cells. They also emphasize the great amount of training, experience

26. Kraushaar, O. F., and Bradbury, J. T.: J. Lab. & Clin. Med. **33**:1195, 1948.

27. Birge, R. F.; McMullen, T., and Davis, S. K.: Am. J. Clin. Path. **18**:754, 1948.

28. Woolner, L. B., and McDonald, J. R.: J. A. M. A. **139**:497, 1949.

29. Pollard, H. M.; Bryant, H. C.; Block, M., and Hall, W. C.: J. A. M. A. **139**:71, 1949.

and time required for the successful use of this method of diagnosis. Griffin [30] stressed that point. Fremont-Smith, Graham and Meigs [31] stated, "The ability to make accurate diagnoses by this method is difficult to acquire, obtainable only by months of intensive training and perfected only through constant use." Caution is expressed lest workers lacking the broad training and experience cited attempt to employ this method.

With these statements and cautions we have no argument; we agree that they are basically sound. We note, however, that increasing numbers cite their experience with technics more closely resembling those used in the general practice of pathology. Hunter and Richardson [1] have emphasized the advantages of blocking and sectioning this material. In the procedure we have outlined, no part of the diagnosis is relegated to technicians or "cytologists." Some experience is necessary for the pathologist to assemble the facts with which he is already familiar, to correlate them with the material presented in current literature and to apply the criteria of cancer to this method of examination. For this experience the method of McKay, Ware, Atwood and Harken,[18] in which material removed from the bronchi at the time of autopsy in cases of bronchogenic carcinoma was examined and compared with prepared sections, is to be commended. We believe that by previous training in oncology the pathologist is already basically prepared for this procedure, that by this method no part of the diagnosis is relegated to those with less training and that it is in no way prohibitive economically. Recent reviews of our preparations and of reports made during the past two years and follow-up studies of cases impress us with the fact that we were unnecessarily cautious over a long period.

RESULTS

During a period of twenty-seven months 101 specimens obtained from 81 patients were examined by this method, providing 1.25 examinations (blocks) per patient. Of this group, 24 have been proved to have carcinoma of the bronchi by biopsy of the primary lesion, surgical resection of tissues for biopsy or of the lung for examination, autopsy or by roentgenogram and clinical progress acceptable to all departments concerned as diagnostic of bronchogenic carcinoma. Of these 24 proved cases of bronchogenic carcinoma, bronchial washings were reported suggestive but not diagnostic in 2, strongly suggestive in 3 and diagnostic of cancer in 10. No diagnosis of cancer was reported in 9 cases.

Nine of the last 10 cases of bronchial carcinoma examined have been positively diagnosed by this method.

30. Griffin, H. K.: Am. J. Clin. Path. **18**:330, 1948.

31. Fremont-Smith, M.; Graham, R. M., and Meigs, J. V.: J. A. M. A. **138**: 469, 1948.

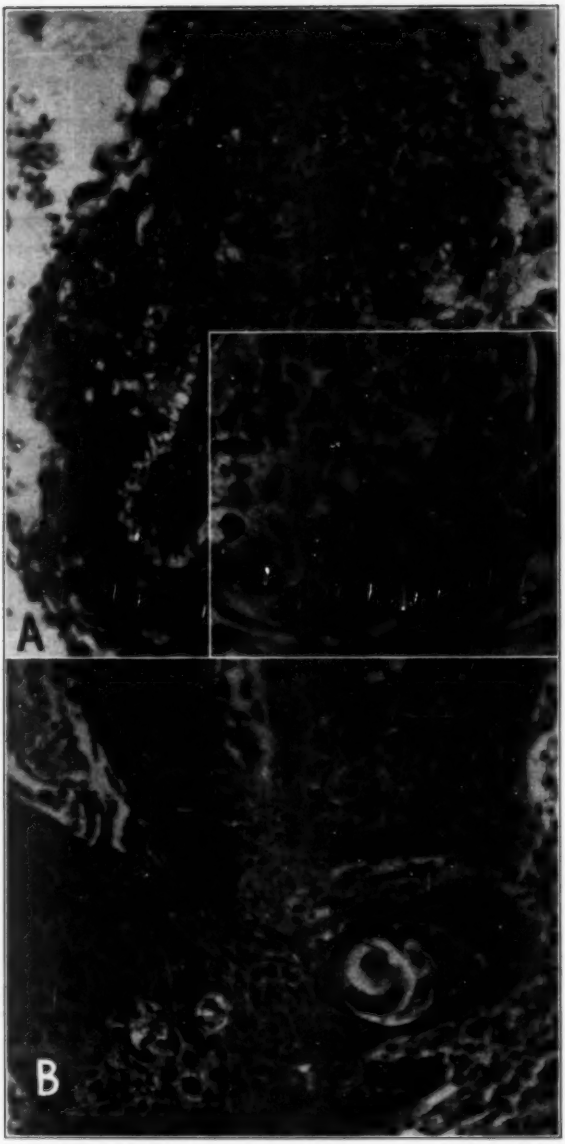


Fig. 1 (case 2).—*A*, section of a bronchial washing showing a group of neoplastic cells. Hematoxylin and eosin; × 200. The insert shows loss of polarity and variations in nuclear size. A mitotic figure is seen at lower center. × 700. *B*, squamous carcinoma of bronchus. Hematoxylin and eosin; × 200.

REPORT OF CASES

CASE 1.—A 48 year old white man entered this hospital because of pain in the left upper region of the chest and hemoptysis of six days' duration. Roentgen examination revealed a homogeneous dense lesion in the upper lobe of the left lung. The sputum contained no acid-fast bacilli. Bronchoscopic examination failed to disclose a neoplasm. Bronchial washings showed cell groups which were suspected of being carcinoma. The clinical findings were not considered diagnostic of cancer, but the lesion persisted, and repeated roentgen studies, including angiography, contributed suggestive but not conclusive evidence of a neoplasm. Thoracotomy was offered but refused, and the patient was discharged, only to be readmitted three months later with essentially the same complaints. The lesion had not diminished in size, as shown by roentgenogram. A biopsy gave negative results, but bronchial washings again showed cell groups which aroused a suspicion of carcinoma. The left lung was resected, and a large neoplasm limited to the upper lobe was found. The pathologic diagnosis was bronchogenic carcinoma, squamous cell type.

CASE 2.—A 50 year old white man was admitted to the hospital, complaining of cough and pain in the chest of four weeks' duration. Roentgenograms of the chest revealed an infiltrative process, thought to be inflammatory, in the upper lobe of the right lung. The sputum contained no acid-fast bacilli or fungi. Bronchoscopic examination revealed a few raised areas at the orifice of the bronchus of the upper lobe of the right lung. "They did not have the appearance of a tumor, but did bleed slightly on manipulation." A biopsy was not made. Histologic sections of bronchial washings were positive for carcinoma (fig. 1 *A*). Pneumonectomy was performed on the right, and a primary neoplasm, which measured 5 cm. in diameter, was found in the upper lobe of the right lung. Histologic sections showed it to be a moderately well differentiated squamous cell carcinoma (fig. 1 *B*). The patient died ten weeks after operation. At autopsy no metastases were found.

CASE 3.—A 53 year old white man was admitted for the diagnosis of a lesion which had been found in the left upper lung field, on routine roentgen examination of the chest at another hospital. He had no symptoms referable to the lungs. There was dulness to percussion over the left upper lung field with diminution of tactile and vocal fremitus and distant breath sounds. Bronchoscopic examination revealed narrowing of the left main bronchus, thought to be due to an extrinsic lesion of the upper lobe. No endobronchial lesion was seen, and no specimen was obtained for biopsy. The bronchial washings were considered diagnostic of carcinoma. The left lung was resected, and a large primary neoplasm was found in the upper lobe. The pathologic diagnosis was adenocarcinoma of the bronchus of the left upper lobe.

CASE 4.—A 50 year old white man was admitted for the fifth time because of a "brassy" cough of several years' duration and recurrent pain in the anterior part of the chest. Roentgen examination revealed that the heart and lungs were within normal limits. The bronchoscopic diagnosis was "asthmatic tracheobronchitis, severe." Prepared bronchial washings yielded many large groups of carcinoma cells in sections (fig. 2 *A*). Further roentgen examinations of the chest and planigraphic studies of the trachea and the main bronchi failed to reveal evidence of an intrinsic or an extrinsic lesion. Additional bronchoscopic examinations made over a period of two months finally showed a slight heaping up of tissue with narrowing of the lumen of the right main bronchus. Biopsy of this tissue revealed a poorly differentiated carcinoma (fig. 2 *B*). Pneumonectomy was done promptly on the right side (fig. 3) and histologic sections of the right main bronchus revealed an early primary squamous cell carcinoma with peribronchial extension only.

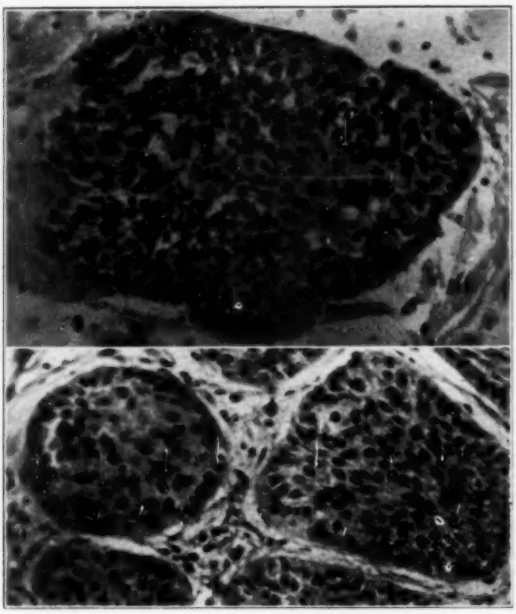


Fig. 2 (case 4).—*A*, sections of bronchial washings of neoplastic tissue. × 200. *B*, biopsy specimen showing poorly differentiated carcinoma. Hematoxylin and eosin; × 200.

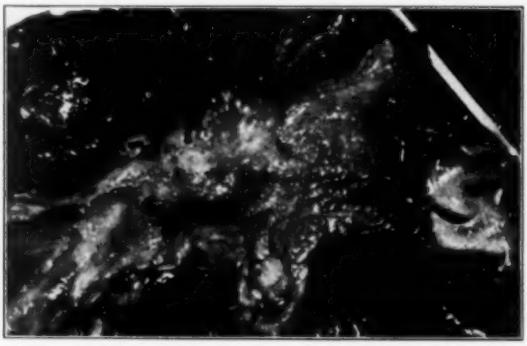


Fig. 3 (case 4).—Squamous carcinoma of the right main bronchus. A stick has been placed along the line of resection. Note that there are thickening and granularity for a distance of 5 cm. in the center of the photograph.

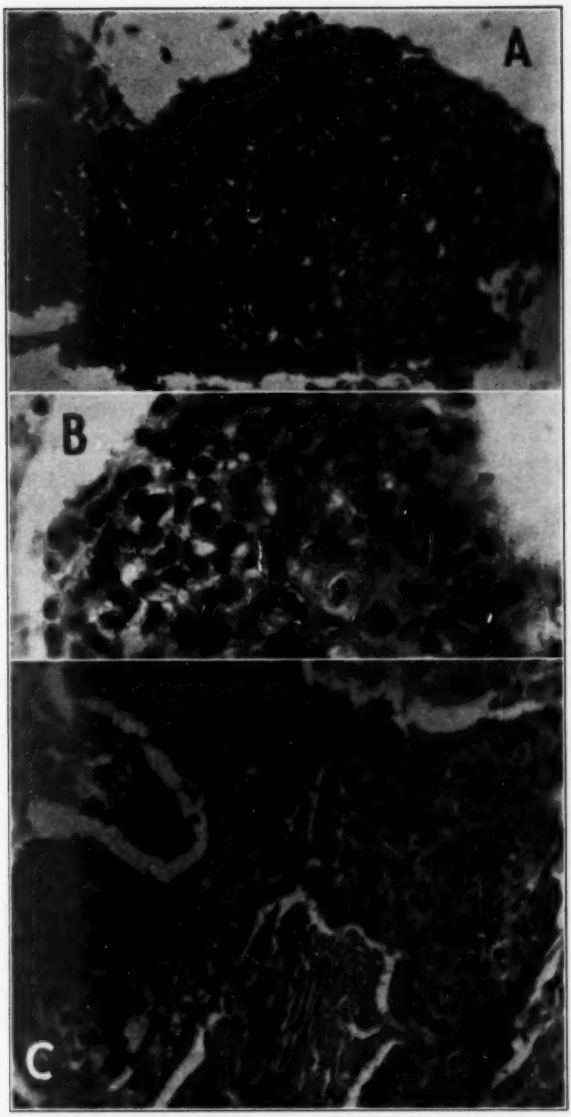


Fig. 4 (case 5).—*A*, section of bronchial washing containing a large island of neoplastic tissue. Hematoxylin and eosin; × 200. *B*, field from *A* showing loss of polarity, variations in size, contours and staining intensity of nuclei, and mitoses. Hematoxylin and eosin. × 700. *C*, section of squamous carcinoma of the bronchus of the upper lobe of the right lung, found at autopsy. Hematoxylin and eosin; × 350.

CASE 5.—A 60 year old white man complained of painful joints and varicose veins. The roentgenogram presented a right hilar shadow which was interpreted as probably bronchogenic carcinoma. On bronchoscopic examination a mass was observed in the bronchus of the upper lobe of the right lung. Because of its position, a good biopsy specimen could not be obtained. Sections of the bronchial washings were considered extremely suggestive of cancer (figs. 4 *A* and *B*). Pathologic examination of the tissue revealed bronchial mucosa that was reported to be suggestive, but not diagnostic of cancer. Exploratory thoracotomy was performed and an inoperable lesion of the upper lobe of the right lung was found. At autopsy, histologic sections of the lesions of the right upper lobe bronchus revealed a primary, poorly differentiated epidermoid carcinoma (fig. 4 *C*).

It is of interest to note that this case was seen early in our series. Caution at that time is illustrated in the diagnosis of the aspirated material as suggestive of cancer.

SUMMARY

Follow-up studies to date demonstrate that our policy and procedure are effective in that carcinoma of the bronchus has been detected early in a reasonably high percentage of cases. Our failures to identify cancer cells occurred principally during the first half of the period reported. A review of our past material at this time indicates that skepticism as to the value of the procedure and unnecessary caution not only lowered the percentage of diagnoses but delayed appreciation and adoption of the procedure as a valuable diagnostic aid.

Aspirated material and/or washings obtained through the bronchoscope constitute a valuable source of cancer cells, particularly as cell groups, for the diagnosis of bronchogenic carcinoma. This material, after centrifugation, can be fixed, embedded, sectioned and stained just as any other biopsy material. From our experience and a review of current literature we conclude that the examination of aspirated bronchial material and/or washings constitutes an aid to the early diagnosis of bronchogenic carcinoma of such great value that its use can no longer be ignored by the general pathologist.

ACCLIMATIZATION RESPONSE AND PATHOLOGIC CHANGES IN RATS AT AN ALTITUDE OF 25,000 FEET

BENJAMIN HIGHMAN, M.D.
AND
PAUL D. ALTLAND, Ph.D.
BETHESDA, MD.

A STUDY of the growth and reproduction of rats exposed to a high altitude for four hours daily has been reported by one of us (Altland [1]). In the course of that study it was found, as will be reported here, that such exposures produced certain acclimatization responses, often severe changes in the heart and other organs, and premature death. Other investigators [2] have studied the organic changes associated with acute high altitude hypoxia and short term continuous hypoxia, but the nature of the physiologic adjustments and the severity of the pathologic changes encountered in these long term experiments have not been reported previously.

METHODS

The procedure of exposure was essentially the same as that previously described.[1] One hundred and seventy-nine male and 130 female Sprague-Dawley rats were exposed to 25,000 feet of simulated altitude four hours daily, starting at 14 days of age. Of these, 79 males and 60 females were killed for study, 59 males and 36 females died in the altitude chamber during exposure, and 41 males and 34 females were found dead in their cages during intervals of rest. All rats were examined as soon as possible, usually within four hours after death, but a delay of as long as twelve to eighteen hours was unavoidable with some rats that died at night.

The tissues saved for histologic examination were generally fixed in a buffered solution of formaldehyde. The bones were decalcified with 5 per cent formic acid. Paraffin sections were stained routinely with azure eosinate,[3] with the

From the Laboratory of Pathology and Pharmacology (Dr. Highman) and the Laboratory of Physical Biology (Dr. Altland), Experimental Biology and Medicine Institute, National Institutes of Health.

1. Altland, P. D.: J. Exper. Zool. **110**:1, 1949.

2. Campbell, J. A.: (*a*) J. Physiol. **62**:211, 1927; (*b*) ibid. **63**:325, 1927; (*c*) Brit. J. Exper. Path. **16**:39, 1935. (*d*) Armstrong, H. G., and Heim, J. W.: J. Aviation Med. **9**:45, 1938. (*e*) Sundstroem, E. S., and Michaels, G.: Univ. California Publ. **12**:1, 1942. (*f*) Dalton, A. J.; Jones, B. F.; Peters, V. B., and Mitchell, E. R.; J. Nat. Cancer Inst. **6**:161, 1945. (*g*) Reynolds, O., and Phillips, N. E.: Am. J. Physiol. **151**:147, 1947. (*h*) Lewis, R. B., and Haymaker, W.: J. Aviation Med. **19**:306, 1948.

3. Lillie, R. D.: Histopathologic Technic, Philadelphia, The Blakiston Company, 1948.

acidulated ferrocyanide reaction for iron and with the Dunn-Thompson modification of Van Gieson's stain for hemoglobin,[4] and occasionally, when indicated, with Masson's trichrome and other special stains. Frozen sections of heart, liver, kidney and adrenal gland were stained for fat with oil red 0.[5]

The organ weights of exposed rats killed for study were compared with those of control rats of equivalent body weight. This was necessary since the exposed female and male rats weighed 21 and 36 per cent less than controls of corresponding ages.[1]

Tail blood samples were obtained at regular intervals and analyzed for percentage of packed red cells and for hemoglobin content. The hematocrit value was obtained by using Van Allen tubes and centrifuging at 2,000 revolutions per minute for thirty minutes. Oxyhemoglobin concentration was determined

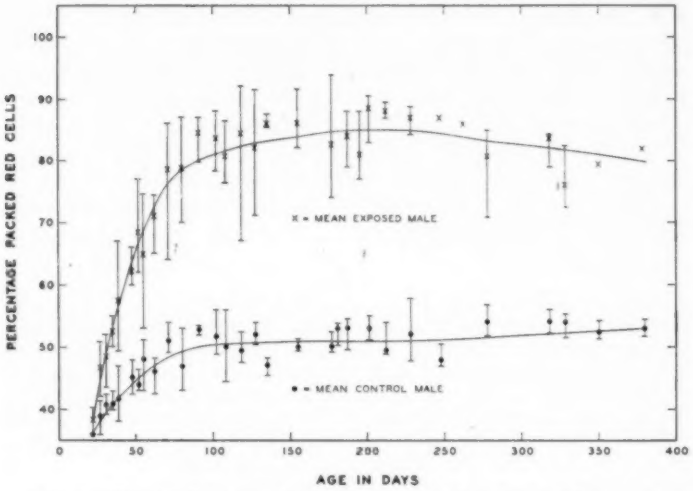


Fig. 1.—Hematocrit values of male rats exposed four hours daily to a simulated altitude of 25,000 feet. Maximum and minimum values are given for various ages.

with a Beckman spectrophotometer by using extinction coefficients reported by Horecker.[6] Measurements were taken at wavelengths of 5100, 5400, 5600 and 5765 angstroms to establish whether any pigments other than oxyhemoglobin were present. An average value of these four readings was used to calculate the percentage of oxyhemoglobin.

RESULTS

An important effect of the altitude to which the rats were daily exposed was the development of polycythemia. The hematocrit and oxyhemoglobin values

4. Dunn, R. C., and Thompson, E. C.: Arch. Path. **39**:49, 1945.
5. Lillie, R. D., and Ashburn, L. L.: Arch. Path. **36**:432, 1943.
6. Horecker, B. L.: J. Biol. Chem. **148**:173, 1943.

of both sexes began to increase gradually during the second week of exposure and continued to rise until a plateau was reached between 70 and 100 days (fig. 1 and 2). Although there was some individual variation, the mean values of the hematocrit and the oxyhemoglobin concentration were 39 and 35 per cent, respectively, above control levels. It is noteworthy that these high levels persisted for the duration of the experiment in most of the rats which maintained their body weight. The mean count for 20 exposed rats over 90 days old was 10,235,000 erythrocytes per cubic millimeter (range, 8,500,000 to 11,200,000) while that of a similar number of controls was 8,000,000 (range, 7,550,000 to 8,450,000). The leukocyte count of these exposed rats was not increased. Analysis of smears of the femoral marrow of 10 of the exposed rats showed an absolute increase of the precursors of erythrocytes and an absolute decrease of those of leukocytes. (The data on the marrow smears and the blood counts of these rats were furnished by Dr. K. M. Endicott.) The number of megakaryocytes seen in sections of the marrow was not significantly altered. Thrombocyte counts were made on 6 additional exposed rats and 5 controls. The counts of 3 exposed animals were normal and those of the other 3 were decreased. All 6 exposed animals showed polycythemia.

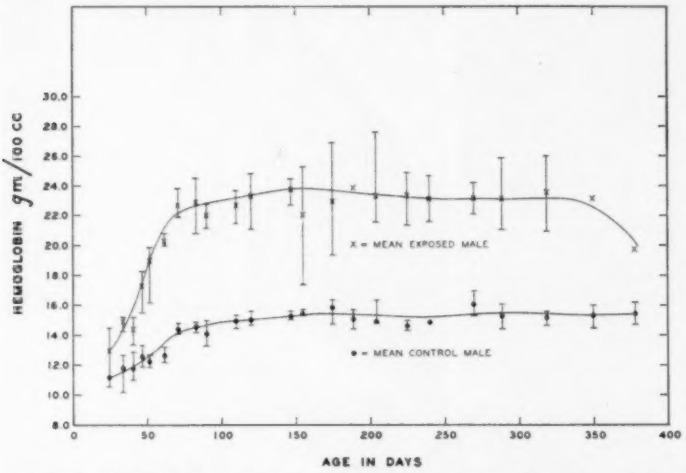


Fig. 2.—Hemoglobin concentrations of male rats exposed four hours daily to a simulated altitude of 25,000 feet. Maximum and minimum values are given for various ages.

The ability of the animals to withstand daily exposure to a simulated altitude of 25,000 feet varied greatly. Approximately 30 per cent of the rats died during the first 100 days, 65 per cent were dead by the end of 200 days, and only 10 per cent survived more than 300 days. There was little difference in the ability to withstand the stress between the males and the females. The oldest female reached 433 days of age and the oldest male 400 days, or less than half of the normal life span of this strain of rats. Some rats died unexpectedly, usually in the altitude chamber, without warning signs or symptoms; in other instances

death was preceded by a reduction of the activity of the rat associated with a loss of body weight and often a drop of blood values.

PATHOLOGIC OBSERVATIONS

Altogether, the tissues of 38 control rats (nonexposed), 47 exposed rats killed for study and 61 exposed rats that died during the experiment were studied histologically and comparisons made according to age and sex. Pathologic changes occurred in all the exposed rats, but severe changes were generally more common in the exposed rats that died than in those that were killed. One of the most constant findings in exposed rats was gross hypertrophy of the heart, with an increase of its weight. There was considerable individual variation in the relative

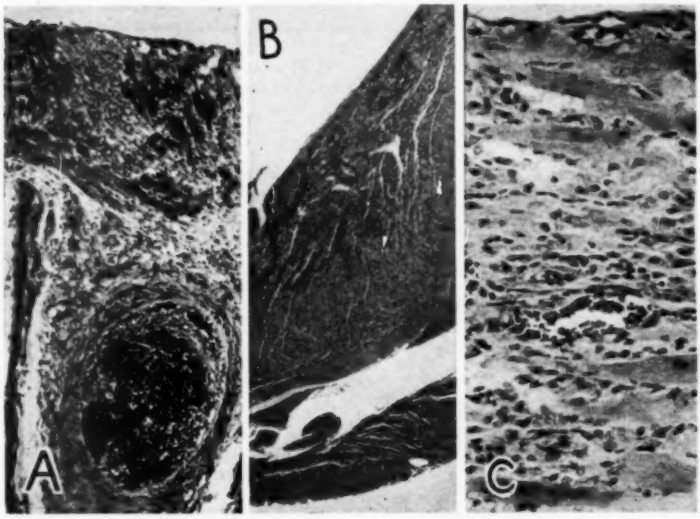


Fig. 3.—*A*, section of the heart of a rat 119 days old, showing extensive infarction of the myocardium with a prominent inflammatory reaction. Note the organizing thrombus in the large artery lying between the atrium and the right ventricle. The epicardium is in the upper portion of the figure. Masson's trichrome stain; × 70.

B, section of the heart of a rat 101 days old, showing fibrous changes in the myocardium around the left ventricle but not around the right ventricle. Azure eosinate; × 12.

C, higher power view of area of left ventricle shown in *B*. Note preservation of muscle fibers immediately beneath the endocardium. Azure eosinate; × 200.

gain in cardiac weight but there was no significant difference in this respect between the sexes or between the age groups. The chambers were frequently dilated and their walls thickened. The average diameter of 20 or more fibers in the interventricular septum was determined and was found to exceed 14 microns in 52 of 92 exposed rats and in only 2 of 36 controls.

One male rat found dead at 119 days had infarction of most of the myocardium and organizing thrombi in two large and several small arteries at the base of the heart (fig. 3 *A*). A male rat dying at 168 days showed subtotal scar tissue replacement of the interventricular septum. In both of these rats there were vegetations on the aortic valve (described in a later paragraph). Six additional rats showed areas containing many scattered, poorly staining, atrophic or necrotic muscle fibers. Slight myocardial fatty degeneration was seen in 8 of 42 males and in 1 of 25 females over 100 days of age, and several rats had small hemorrhages in the myocardium. In about half of the exposed rats there was a relative increase in the fibrous tissue with disappearance of many muscle fibers in the inner third of the myocardium surrounding the left ventricle (fig. 3 *B*). This occurred less frequently in the septum and near the apex and occasionally elsewhere. Generally such areas showed numerous fibroblasts and mononuclear cells, often a few phagocytes laden with fat or hemosiderin, and occasionally a few atrophic, fatty or frankly necrotic muscle fibers. The muscle fibers immediately beneath the endocardium were usually preserved (fig. 3*C*).

The endocardial changes were confined chiefly to the valves (fig. 4). The majority of the exposed rats had slight to marked irregular or nodular thickening of the valves, usually most impressive in the distal portion. The changes were more frequent and severe in the mitral than in the aortic and tricuspid valves. The maximum thickness of some valves was more than five times that of the controls. The thickened valves often showed marked cellular proliferation and focal areas of edema, mucoid degeneration, hemorrhage and, chiefly in older rats, fibrosis, hyalinization, necrosis or calcification. Capillaries were numerous in some of the thickened valves and occasionally extended to the free edge. Organizing vegetations of variable size were seen along many of the thickened mitral and aortic cusps and on the cusps of one tricuspid valve. The incidence increased with age; vegetations were seen in only 1 rat under 100 days of age and in about half of the rats over 300 days. They occurrel chiefly near the line of closure and along the upper surfaces of the valves. Phagocytes laden with brown pigment, chiefly hemosiderin, were seen submarginally in some valves, and fat-laden phagocytes were frequent around necrotic foci and at the base of vegetations. Some vegetations were partially covered by endothelium; a few contained small groups of neutrophilic leukocytes, and a few showed occasional basophilic granules and small deposits of calcium. The mitral valve of a rat that died at 349 days of age was densely infiltrated by neutrophils in some areas and showed many large colonies of gram-positive and gram-negative micro-organisms along the borders of a large vegetation. No organisms were found in other tissues of this rat. A similar vegetation with bacterial colonies was seen on the tricuspid valve of a 433 day rat. Gram and fibrin stains made on the cardiac valves of a number of other rats revealed fibrin but no bacteria in the vegetations.

The auricular endocardium occasionally showed focally slight cellular proliferation, fibrous thickening, ulceration or calcification. The auricles of 6 of 60 rats contained unorganized and organized vegetations, some of these showing a little hemosiderin and calcium.

The epicardium of a few animals was thickened and moderately infiltrated by monocytes. This condition was usually associated with either pleuritis or some adjoining myocardial lesion. Two rats showed epicardial hemorrhages overlying necrotic areas.

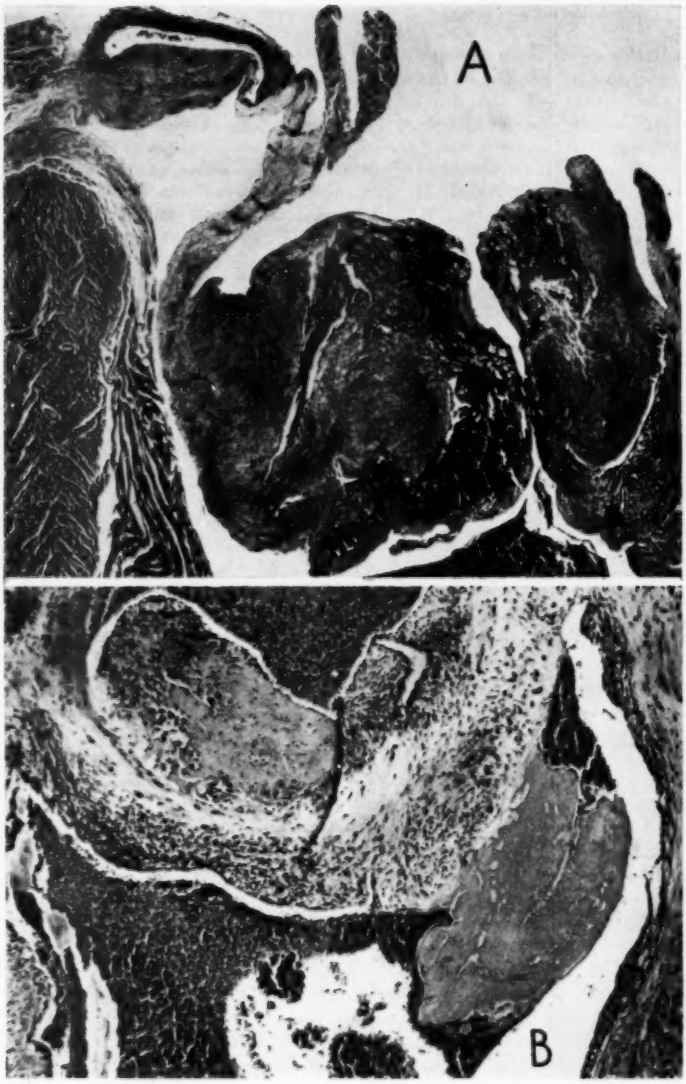


Fig. 4.—*A*, section of the heart of a rat 117 days old, showing distinct thickening of the mitral valve with large vegetation. Van Gieson stain as modified by Dunn and Thompson; × 20.

B, Section of the heart of a rat 309 days old, showing vegetations on the base of the aortic valve. Note that the upper vegetation is organized and partially covered by endothelium. Azure eosinate; × 50.

There was considerable variation in the weight of kidneys of exposed rats. Nearly all showed notable capillary congestion. The average diameter of the glomeruli, estimated by measuring 20 to 50 in each kidney, exceeded 130 microns in 49 of 95 exposed rats and in only 4 of 34 unexposed controls. Since tangential sections of glomeruli were included, the actual average diameter is higher than the estimated average.

Nineteen of 80 rats over 100 days old and one 60 day rat showed single or multiple infarcts (fig. 5 *A*) of one or both kidneys, occasionally involving nearly an entire kidney. There was no striking difference in incidence between the sexes and different ages. The infarcts appeared triangular in sections with a thin subcapsular layer of viable parenchyma along the base. Nearly all were of the anemic type, but a few were hemorrhagic throughout or showed a hemorrhagic border, sometimes infiltrated focally by neutrophils. In older rats the infarcts often showed tubular and glomerular atrophy, extensive fibrosis, hyalinization and calcification, and definite shrinkage with depression of the overlying capsular surface. In a few of the kidneys there were small wedge-shaped cortical scars suggesting healed infarcts. Several kidneys had thrombosed vessels in infarcted areas. One showed recanalization of a thrombus in a medium-sized artery in a noninfarcted area, and one had a thrombotic mass, possibly an embolus, in a small artery near an infarct. Numerous neutrophils, but no bacteria, were seen in the thrombus and in and around the vessel wall. Another kidney, with a recent and a focally calcified old infarct, had a similar clot in a large pelvic artery at a point where the lumen was narrowed by a large hemosiderotic fibrotic nodule. This nodule resembled an old organized thrombus or embolus. The renal infarcts were often associated with thickening and vegetations of the valves of the heart. Many of the infarcted kidneys also showed slight to moderately extensive focal or segmental fatty changes in the muscular coat of the large and medium-sized arteries (fig. 5*C*). Such changes were occasionally present in noninfarcted kidneys but were not seen in the heart, the liver or the adrenal glands.

Marked hemosiderosis of the renal cortex was seen in nearly all rats after ten weeks' exposure. Heavy deposits were seen along the borders of infarcts and in scattered clusters of convoluted tubules, and lesser amounts in intervening tubules. The pigment granules were most prominent at the base of the epithelial cells and ranged in size from barely visible to more than 5 microns. They were seen occasionally in intratubular desquamated cells, in fibroblasts and capillary endothelial cells and rarely in the epithelium of medullary tubules.

Small numbers of scattered tubular hemoglobin casts (usually less than 10 per section) were frequent in both control and exposed rats. Numerous hemoglobin casts (from 35 to over 100 per microscopic section) were seen in 9 of 53 exposed rats but in none of 16 controls between 100 and 300 days of age and in 3 of 11 control rats over 300 days. In addition, some exposed rats showed hyaline casts and pigmented casts, some of the casts staining in part like hemoglobin.

About 33 per cent of the males and 46 per cent of the females over 100 days had a slight to moderate increase of lipoid material in the kidney. The lipoid material was seen chiefly in the epithelium of scattered small groups of cortical tubules and along the borders of infarcts. Several kidneys disclosed fibrosis, hyalinization and less severe changes in many glomeruli and epithelial desquamation, hyaline droplets and other changes in many tubules. Variable numbers of small calcareous deposits were seen in several exposed and 1 control animal, chiefly at the junction of cortex and medulla, and often gave the prussian blue reaction for iron.

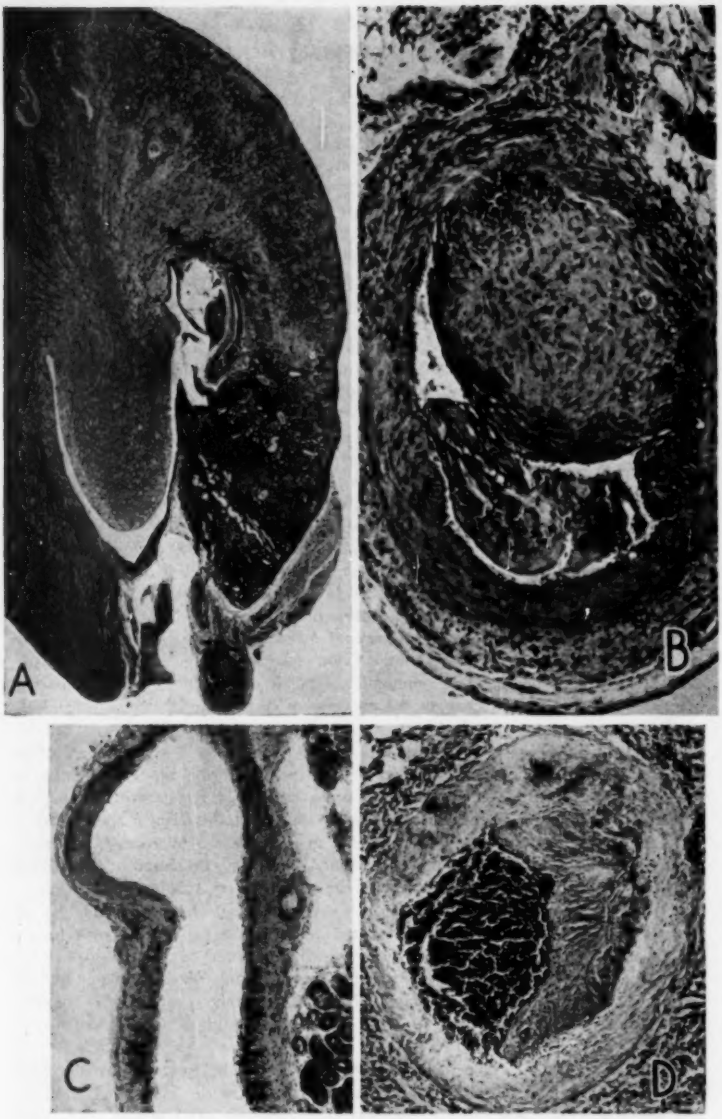


Fig. 5.—*A*, section of a kidney of a rat 309 days old, showing a large recent infarct above, a smaller, older infarct below and a thrombosed artery at the bottom of the section. Azure eosinate; × 10.

B, higher power view of the artery in *A*. Note the fibrous nodule above and the recent thrombus below, free in the lumen of the artery. Azure eosinate; × 100.

C, frozen section of a kidney of a rat 298 days old. Note focal fatty changes (dark areas) in the media of the large artery. Many tubules show dark fat droplets and lighter (gray) hemosiderin granules. Oil red 0; × 60.

D, section of a lung of a rat 332 days old, showing a large vessel with extensive fibrous thickening and focal calcification of the intima and a large subendothelial fibrinous plaque. Masson's trichrome stain; × 15.

Gross hematuria, usually confirmed microscopically, was observed in 6 males and 7 females between 79 and 226 days of age. Ten of these rats died shortly thereafter. In 1 of the 3 that survived, the hematuria was followed by a temporary sharp drop in blood count, hematocrit reading and hemoglobin value, and autopsy 177 days later revealed an old renal infarct. The 2 other rats that survived after hematuria later revealed small hemosiderotic cortical scars suggesting healed infarcts. The kidneys of 4 rats with terminal hematuria were studied histologically. All had distinct congestion and hyaline droplets or other severe degenerative changes in the renal convoluted tubules; 3 showed focal fatty changes in the media of some of the larger blood vessels, 1 a recent thrombus in a large artery, and 1 an area suggesting early infarction.

The average weight of the spleens of rats exposed over 105 days was 24 per cent more than that of the controls. Erythropoiesis was on the whole much more appreciable in the spleens of exposed rats under 200 days than in those of controls of a similar age. Hemosiderin was greatly reduced in amount or absent in nearly all rats under 100 days, and was relatively small in amount in males thereafter, but approached normal in females over 200 days. Many exposed rats, particularly the males, also showed a slight to marked decrease in the average size of the malpighian corpuscles and in the number and the size of the germinal centers. The perifollicular zone of pale reticulum cells was often narrowed and poorly demarcated.

Controlobular congestion with narrowing of the liver cell cords was seen in nearly all the exposed rats and was severe most frequently in those between 100 and 200 days of age. Slight to moderate hemosiderosis of Kupffer cells, rare in controls, was seen in about 20 per cent of exposed males and 50 per cent of exposed females over 100 days old. About 20 per cent of exposed males and females over 100 days old had slight to moderate fatty changes in the liver cells.

Severe intestinal hemorrhage with massive accumulation of blood occurred in both males and females and was found in 26 per cent of all rats that died during the experiment. In many cases the entire intestine was engorged with blood. The cecum and adjoining gut were most frequently affected. This condition was found after 7 weeks of exposure (age, 65 days) and as late as the forty-ninth week (age, 363 days) but was most prevalent between the third and the ninth month of exposure. Gross examination of the intestinal mucosa revealed no clearly recognizable localized source of the bleeding. The intestines of 8 of these rats were examined microscopically. All showed severe congestion and increased vascularity throughout, with extensive disorganization and hemorrhages in the mucosa. Three showed ulceration of the mucosa, with polymorphonuclear leukocytes infiltrating the subjacent submucosa and, in 2 rats, the muscularis. A few leukocytes were seen focally in other areas.

The stomachs of the rats with blood in the intestinal lumen were without exception devoid of blood. With the dissecting microscope small gastric ulcers were seen in the stomachs of 5 of 79 exposed rats. Histologic study of one of these gastric ulcers revealed an irregular base of dense fibrous tissue covered by fibrinoid material admixed with nuclear debris and a few leukocytes. The muscularis mucosae was interrupted, and the underlying submucosa was markedly thickened and showed numerous capillaries, fibroblasts and mononuclear cells, and occasional hemosiderin-laden phagocytes. A small artery in the submucosa near the ulcer showed marked hyaline thickening of the intima.

The lungs of the exposed animals were often strikingly congested, and the majority showed slight to moderate hemosiderosis of the septal cells and of

occasional alveolar phagocytes. In a few rats the hemosiderin was confined to a portion of one lobe, suggesting a previous hemorrhage.

A female rat that died at 295 days showed purulent bronchitis and consolidation of one lobe with extensive atelectasis, interalveolar fibrosis and areas of organizing pneumonia and hemorrhage. Many alveoli were lined by cuboidal type cells, many were filled with large mononuclear cells heavily laden with hemosiderin, and some contained elongated brown crystals staining like hemoglobin. In some hemorrhagic areas the alveolar septums were not clearly seen, suggesting infarction.

In the lung of another rat, killed at 332 days, there were numerous large vessels showing marked irregular thickening of the intima (fig. 5*D*). Along the lumen were massive plaques, some partially covered by endothelium, formed of hyaline eosinophilic material staining like fibrin, admixed with nuclear debris and a few scattered mononuclear cells. In areas not covered by endothelium the plaques were often marginated with leukocytes. The intima was formed of moderately cellular, dense fibrous tissue which often showed areas of hyalinization, necrosis and calcification. The media was usually thin and frequently scarred. In a few vessels it contained striated muscle fibers (pulmonary veins). The adventitia was often inconspicuous. Such vascular changes were not seen in the other organs. The heart of this rat revealed marked thickening of the mitral valve with organized vegetations in the left auricle.

A few rats showed pulmonary emphysema and a few focal calcification of pulmonary vessels, but the incidence of edema, hemorrhages, atelectasis and pneumonia was not significantly increased in the exposed animals.

The thymuses of exposed rats weighed from 5 to 77 per cent (mean, 40 per cent) less than those of controls, but the nature of the cyclic development and regression of this gland during the first four months of life makes comparison difficult. Nearly all the thymuses studied were markedly congested. Thymic hemorrhages occurred frequently in exposed rats under 200 days that were found dead and occasionally in older rats. The incidence and the degree of cortical atrophy were relatively greater in exposed rats under 200 days than in controls of similar age. In older rats the incidence was about the same.

The cervical lymph nodes of both sides of 9 exposed rats 94 days old were removed and weighed wet. The mean weight of the pooled nodes was 69 mg. (range, 30 to 139 mg.), compared with a mean weight of 175 mg. (range 170 to 180 mg.) for those of controls. The lymph nodes varied considerably histologically, some differing little from those of corresponding controls. In general, they showed a considerable reduction in the number of primary follicles and in the number and the size of the germinal centers. Often the medulla revealed a relative increase in size and loose structure with wide sinusoids, and occasionally it contained fatty tissue. Hemorrhagic extravasations and a variable amount of phagocytosed hemosiderin were seen in the nodes of a few older rats found dead after exposure. Examination of the vertebrae and the femurs of these 9 exposed rats disclosed slight to considerable congestion of the marrow with increased cellularity and absence of fat. The smaller vessels of the meninges and the spinal cord were dilated and engorged.

The spinal cords and brains of 15 animals were examined and showed striking vascular engorgement throughout. One rat killed for study at 391 days had a tumor of the pons, probably a glioma. This tumor is considered to have been an incidental finding, unrelated to the hypoxia, and will be reported in detail in a separate paper.

Partial or complete paralysis and cyanosis of the hindlimbs was observed in a male aged 217 days and in 3 females aged 98, 124 and 128 days. These symptoms appeared suddenly, preceded only by a slight loss of weight, and culminated in death from 1 to 4 days. During this period the rats moved about by propelling themselves with their forelimbs. The blood values of these rats remained high; one such rat had a hematocrit reading of 90 per cent and a hemoglobin concentration of 26 Gm. per hundred cubic centimeters. one day before death. The 217 day old male had blood in the subarachnoid space, the cerebral ventricles and the central canal of the spinal cord. In the 128 day female there was an extradural hemorrhage dorsal to the spinal cord with a slight lymphocytic infiltration of a dorsal ganglion and adjacent spinal nerve roots. This infiltration is considered to have been an incidental finding.

The findings in the reproductive system and the adrenal glands have been detailed elsewhere.[7] In general, the testes showed premature sloughing of spermatids and spermatocytes, and thus a marked reduction of the number of spermatozoa was effected. These sloughed cells were abundantly present in the lumens of the epididymis. The ovaries showed no structural abnormalities, and cyclic estrus was not usually disturbed, but gestation was affected to such a degree that no living young were produced.

The adrenal glands generally showed slight to distinct congestion, with widening of the sinusoids, particularly in the outer part of the zona reticularis, occasionally grading into necrosis. The cortex was thickened in most exposed rats over 100 days, and in at least some the diameter of the medulla was considerably increased. Slight to moderate hemosiderosis of the vascular endothelium was frequent, particularly in females. The concentration of lipoid material was often reduced, particularly in the zona glomerulosa of exposed rats over 300 days old.

COMMENT

Our findings indicate that severe cardiac damage may result from repeated exposure to high altitude. Hypertrophy and fatty degeneration of the heart have been described by other investigators, but the occurrence of infarction, fibrosis and other histologic evidence of severe myocardial damage, such as that reported herein, apparently has not been reported in previous altitude studies.[8] Thickening of the atrioventricular valves has been reported by Dalton and co-workers [2f] in rats exposed twelve weeks, but the changes were apparently less frequent and severe than in our study. Moreover, he found vegetations only on the mitral valve. As a group, the cardiac vegetations in our rats appeared sterile. The incidence of infected vegetations approximated that of the bacterial endocarditis found by Wilens and Sproul [9] to occur spontaneously in the rat.

7. Altland, P. D.: Proc. Pennsylvania Acad. Sc. **22**:35, 1948; footnote 1.

8. Van Liere, E. J.: Anoxia: Its Effect on the Body, Chicago, University Chicago Press, 1942. Van Liere, E. J.: Am. J. Physiol. **116**:290, 1936. Campbell, J. A.: Brit. J. Exper. Path. **8**:347, 1927. Thorn, G. W.: Clinton, J., Jr.; Farber, S., and Edmonds, H. W.: Bull. Johns Hopkins Hosp. **79**:59, 1946. Campbell.[2a] Armstrong and Heim.[2d] Sundstroem and Michaels.[2e] Dalton and others.[2f] Reynolds and Phillips.[2g] Lewis and Haymaker.[2h]

9. Wilens, S. L., and Sproul, E. E.: Am. J. Path. **14**:177, 1938.

The frequent occurrence of thrombosis, infarction, hemorrhages and peptic ulcer in polycythemia vera[10] (primary polycythemia) suggests that secondary polycythemia may have been responsible in part for similar findings in our animals. However, in contrast to the usual findings in primary polycythemia, our exposed animals had a low or normal platelet count and severe changes in the heart and other organs. It is possible that some of the infarcts were caused by embolism. This is suggested by the frequency of cardiac vegetations in animals showing renal infarcts. Moreover, in each of the 2 rats presenting extensive cardiac infarction and septal fibrosis, respectively, there were present on the aortic valve large vegetations which could have been a source of emboli. The possibility that some of the infarcts may have been due to air embolism must also be considered, but the low incidence of infarcts in animals under 100 days suggests that this is not a major factor.

Renal infarcts and hematuria have not been reported previously as observed in animals exposed to high altitude, but hematuria has been observed in men suffering from "soroche" in the Andes.[11] Some of the exposed rats in this study showed numerous hemoglobin casts and nearly all showed marked hemosiderosis of the kidney and less frequently of the liver, the lung and the adrenal gland. These findings may be due to excessive hemolysis, since similar changes have been noted in experimental animals following administration of hemolytic agents.[12] It is noteworthy that marked renal hemosiderosis was observed in some rats with a subnormal amount of hemosiderin in the spleen.

Intestinal bleeding appeared to be one of the major contributory causes of death. Similar hemorrhages were found in rats exposed continuously to high altitude by Sundstroem and Michaels.[2e] Gastric ulcers were not as frequent as reported in other altitude studies.[13]

Repeated daily exposure to a simulated altitude of 25,000 feet greatly reduced the life span of rats. This was probably due in many cases to infarcts, hemorrhages and other pathologic changes. The myocardial fibrosis seen in many of our rats resembled that reported

10. Harrop, G. A.: Medicine **7**:291, 1928. Weber, F. P.: Polycythemia, Erythrosis, and Erythraemia, London, H. K. Lewis & Company, Ltd., 1921; Addenda, London, H. K. Lewis & Company, Ltd., 1929. Weber, F. P.: Lancet **227**:808, 1934. Boyd, W.: Am. J. M. Sc. **187**:489, 1934. Reznikoff, P.; Foot, N. C., and Bethea, J. M.: Am. J. M. Sc. **179**:753, 1935. Cecil, R. L.: Textbook of Medicine, Philadelphia, W. B. Saunders Company, 1944, p. 987.

11. Monge, C: Physiol. Rev. **23**:166, 1943.

12. Spicer, S. S.; Highman, B., and Monaco, A. R.: J. Pharm. & Exper. Therap. **95**:256, 1949.

13. Dalton and others.[2f] Reynolds and Phillips.[2g]

by Wilens and Sproul[9] to occur spontaneously only in rats over 400 days old. We found numerous hemoglobin casts in the kidney only in control rats that were over 300 days old. In exposed animals, however, numerous casts occurred in much younger animals. These findings suggest that, in addition to producing specific lesions, hypoxia may hasten the development of lesions common in senile animals.

SUMMARY

Rats were exposed to a simulated altitude of 25,000 feet four hours daily, starting at 14 days of age. Hematocrit readings, oxyhemoglobin concentrations and erythrocyte counts increased gradually during the first 100 days and persisted at high levels. The mortality was high after 100 days of age, and none lived more than half of the normal life span.

Nearly all of the exposed rats had striking vascular engorgement and severe lesions in various organs. The heart was usually distinctly hypertrophied, the valves were often thickened, and vegetations were found principally on the mitral, and less frequently on the aortic and tricuspid valves and in the auricles. In addition, many of the rats showed fibrosis of the inner portion of the wall of the left ventricle and fatty and various other degenerative changes in the myocardium, and one rat presented coronary occlusion with infarction.

Infarcts were frequently found in the kidneys. Many kidneys also showed marked hemosiderosis and a lesser number, numerous hemoglobin casts and fatty changes in the media of some arteries or in the epithelium of some tubules. Some rats showed gross hematuria.

A large proportion of the rats that died had a massive accumulation of blood in the intestine, but only a relatively small number had ulcers in the intestine or the stomach. Hemorrhages were also found in the central nervous system.

These findings indicate that rats are unable to acclimatize fully to short daily exposures to a simulated altitude of 25,000 feet. It is suggested that discontinuous exposure of laboratory animals to simulated high altitudes may be a useful experimental method for the production and study of cardiovascular and other lesions.

EXTRAGENITAL CHORIOCARCINOMA

With Comments on the Male Origin of Trophoblastic Tissues

EDWIN F. HIRSCH, M.D.
CHICAGO

CHORIOCARCINOMA,[1] as originally defined, is a cancerous growth of the trophoblastic Langhans and syncytial cells of the placenta. The tumor, accordingly, is an atypical variant of a pregnancy —or more specifically, a cancerous growth developing from a fertilized ovum. In the fertilization of the ovum a male component is fused with a female, and the union initiates a sequential growth of tissue cells, some of which are destined to form an embryo and others to develop into a placenta. This concept of the origin of choriocarcinoma had general acceptance, without exception, until tissues resembling trophoblast were observed in cancerous teratomas, notably those of the testis. That these tumors contained trophoblastic tissues was disputed until methods were found for demonstrating gonadotropic hormones (Aschheim-Zondek reaction). Then the fluids of patients having cancerous teratoma of the testis with trophoblastic tissues were found to have a high content of these hormones.

Brewer [2] discussed the importance of the chorionic gonadotropin with regard to the diagnosis of testicular tumors and emphasized that the classification remained confused because two varieties of gonadotropic hormone could be present in the urine of the patients, and the significance of each one had not been evaluated properly. He quoted many articles describing the biologic properties and differentiation of these two hormones. The one hormone, the chorionic, is identical with that found in the urine of pregnant women and produces luteinization in the ovarian follicles of test animals. The other hormone, the castrate type, is elaborated by the pituitary gland and occurs in the urine of male and female castrates, in women past the menopause and in elderly men. This hormone stimulates follicle growth but not luteinization in the ovaries of test animals. Chorionic gonadotropin appearing in the urine indicates the presence of biologically active tissues in the host and is the more important in the evaluation of a testicular tumor. Brewer stated also

From the Henry Baird Favill Laboratory, St. Luke's Hospital.

1. Marchand, F.: Monatsschr. f. Geburtsh. u. Gynäk. **1**:513, 1895.
2. Brewer, J. I.: Arch. Path. **41**:580, 1946.

that this hormone is produced by cells of the fetal placenta. In the early phase of pregnancy, when the chorionic gonadotropin appears in the urine, the growth of chorionic tissues greatly exceeds that of the embryo proper. As the tissues of the embryo differentiate, the titer of the urinary gonadotropin decreases. Most authors, according to Brewer, agree that this hormone reaches a peak in the urine by the sixtieth to the eighty-fourth day after the first day of the last menstrual period and then falls sharply. At about this time the Langhans cells of the chorionic tissues begin to decrease. During the remainder of the pregnancy the urinary gonadotropin is low. The tissues of the fetus, accordingly, do not produce the hormone. Supporting this conclusion is the observation that in women with abortion or tubal pregnancy the chorionic tissues survive and grow, despite the death of the embryo, and chorionic gonadotropin continues to be excreted in the urine; and also the observation that in women with choriocarcinoma the urine has high titers of chorionic gonadotropin, although no tissues of an embryo or a fetus remain. The hormone disappears from the urine of these patients when the uterus with the tumor is removed, provided no large metastases are present. Later, when these metastases develop, the hormone reappears in the urine.

Brewer stated that (1) all the evidence obtained in clinical and experimental studies indicates that the chorionic gonadotropin is elaborated by chorionic tissues; (2) the tissues of the embryo proper do not produce the hormone; (3) no other tissues are known to produce this hormone; (4) when the hormone is present in patients with testicular tumors, it is identical with the chorionic gonadotropin found in the urine of pregnant women (and those with choriocarcinoma), and (5) in the patients with testicular choriocarcinoma the hormone is produced by the trophoblastic tissues of the tumor.

Primary choriocarcinoma of the ovary is exceedingly rare. Pick [3] in 1904 described a choriocancerous teratoma of an ovary of a girl aged 9 years and since then, according to a recent summary by Oliver and Horne,[4] 12 other tumors proved to be choriocarcinoma have been reported, to which they added another, making a total of 14. Nine of the 14 tumors occurred in children below the age of 12 years; 2 more in girls 13 and 14 years of age—and pregnancy in association with an ovarian teratoma in the oldest (27 years) of the remaining 3 patients could not be excluded by the author himself.[5] Chorionic gonadotropin tests are not recorded in the first reports of these ovarian tumors. The incidence of the ovarian choriocancerous teratomas is at an age when sex

3. Pick, L.: Klin. Wchnschr. **41**:158, 1904.

4. Oliver, H. M., and Horne, E. O.: New England J. Med. **239**:14, 1948.

5. Sbarcea, J.: Centralbl. f. Gynäk. **63**:936, 1939.

differentiation has not been established and in females that genetically could be males.

Mathieu and Robertson [6] stated that about 200 testicular choriocarcinomas had been reported at that time. Friedman and Moore [7] reported 6.4 per cent choriocarcinomas among the embryonal carcinomas and teratocarcinomas they observed among 922 testicular tumors. These embryonal carcinomas and teratocarcinomas comprised 54 per cent of the total number of testicular tumors. This large number contrasts sharply with the few similar ovarian tumors reported and, of course, does not accurately reflect the actual incidence. Symeonidis [8] and others have emphasized this overwhelming preponderance of testicular over ovarian choriocarcinomas. Symeonidis stated that this fact has general biologic interest which reaches beyond the field of oncology and into that of embryology. This almost exclusive development of trophoblastic tissues in the male gonad, he stated, may be due to the specific ability of the male sex cells to produce this teratoma. He ventured to suggest even further that perhaps the trophoblast of the ovum develops because of influences contributed by the male component in the fertilization process. The age range of the males with these tumors has interest and probably also significance. They occur most often in the third and fourth decades, when full functional activity of the tissues of the testis has been established,[7] not at the prepuberty level.

Another group of choriocarcinomas (teratomas) has been described.[9] These are extragenital tumors, and significantly all, to my knowledge, have occurred in males ranging in age from 13 to 72 years. When these growths were described originally, objections were made to the diagnosis because the presence of true trophoblastic cells was disputed. Later, when large amounts of chorionic gonadotropin were demonstrated in the urine of the patients, the objectors contended that these extragenital tumors were secondary to one primary in a testis not examined, or one of small, even microscopic dimension overlooked in the examination of the testis, or one destroyed and leaving only a scar tissue residue. The number of these again is small. Hirsch, Robbins and Houghton listed 14, to which they added another. These extragenital choriocarcinomas have been described as occurring most frequently in the retroperitoneal tissues, then in the mediastinum, in the urinary bladder and, several, in the brain. The origin of those observed in the retroperitoneal, mediastinal and urinary bladder tissues has been explained on the basis of germ tissue displaced from the urogenital ridge, or the equivalent, an abdominal testis

6. Mathieu, A., and Robertson, T. D.: Internat. Abst. Surg. **69**:158, 1939.

7. Friedman, N. B., and Moore, R. A.: Mil. Surgeon **99**:573, 1946.

8. Symeonidis, A.: Centralbl. f. allg. Path. u. path. Anat. **62**:177, 1935.

9. Hirsch, O.; Robbins, S. L., and Houghton, J. D.: Am. J. Path. **22**:833, 1946.

as designated by Symeonidis. Staemmler [10] found accessory testis tissues with germinal epithelium in the retroperitoneal fat near the origin of the inferior mesenteric artery.

REPORT OF A CASE

A white man, aged 26 years, and married, entered St. Luke's Hospital on July 30, 1948 because of a vague pain of four weeks' duration in the right hip which radiated to the lateral side of the knee. Three weeks before, he began to have a dry, unproductive cough and an occasional pain in the right side of the chest. Later the cough became prdouctive, and the secretions were stained with

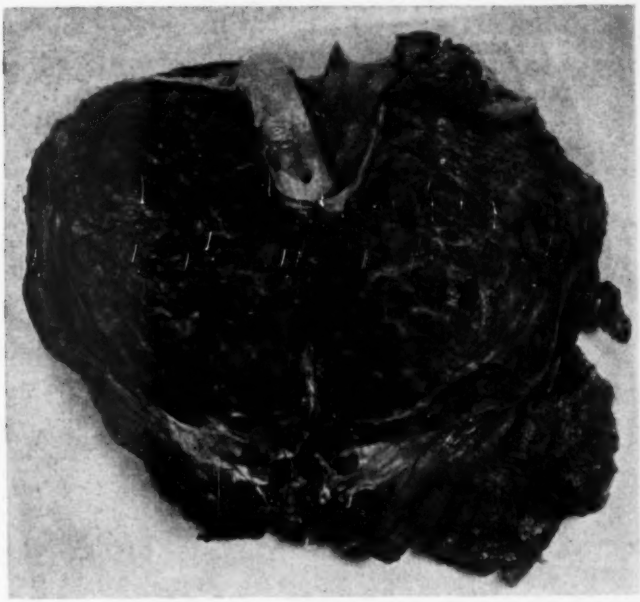


Fig. 1.—Surfaces made by hemisecting the extragenital (retroperitoneal) choriocarcinoma. Note the close proximity of the aorta and the tumor.

blood. He sought medical attention and a roentgen examination of the chest disclosed changes, not specified, in the lungs. A roentgenogram of the hip disclosed nothing. A left orchiopexy had been done in the Army in 1943 for undescended testis. The patient was well nourished. He had a temperature of 99.4 F. The penis was moderately undeveloped, and there was an orchiopexy scar on the left side of the scrotum. The right testis, the size of an almond, was not tender or indurated; the left testis, slightly larger than the right, seemed to have a nontender nodule the size of an orange seed at the inferior pole, connected with

10. Staemmler, M.: Verhandl. d. deutsch. path. Gesellsch. **27**:190, 1934.

the epididymis. The blood had 3,900,000 erythrocytes and 10,150 leukocytes per cubic millimeter, and the hemoglobin content was 10.3 Gm. per hundred centimeters. The alkaline phosphatase was 9.7 units. A roentgenogram of the chest demonstrated extensive metastases in both lung fields, presumably from a testicular tumor.

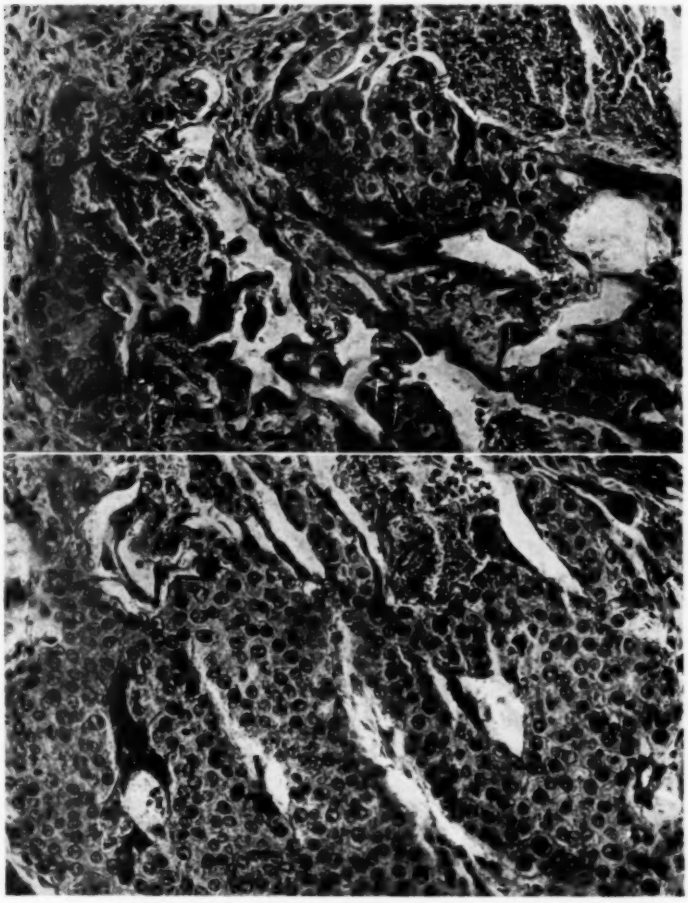


Fig. 2.—Photomicrographs illustrating the large syncytial and trophoblastic cells of the choriocarcinoma; × 198.

An Aschheim-Zondek test was negative at this time. The left testis was removed surgically on August 6, but the gross and microscopic examinations disclosed no tumor. There was distinct hypoplasia of the tubular epithelium. At this time reddish blue nodules appeared in the skin tissues of the left fifth finger, the scalp

and the face. One in the scalp, removed on August 16, was diagnosed as metastatic choriocarcinoma. Shortly after, the patient had a convulsion, considered to be on the basis of a metastasis involving the brain. Hemorrhagic metastases now appeared in the skin of the chest, trunk, arms, hands and face. His anemia became severe, and shortly before death the Aschheim-Zondek test of the urine was positive in dilutions up to 1 to 500. Death occurred on October 12. The postmortem examination of the body was limited to the trunk.

The essentials of the anatomic diagnosis are: primary choriocarcinoma of the right perinephric retroperitoneal tissues; extensive metastatic choriocarcinoma of the lungs, left axillary lymph nodes, diaphragm, stomach, right and left kidneys, verumontanum (colliculus seminalis), small bowel, cecum, scalp and subcutaneous tissues of the body; hypoplasia of the right testis; old orchidectomy scar of the left groin.

Medial to the right kidney and near the midline was a hard mass of tissue 11.5 by 5 by 4.5 cm., its upper edge behind the right renal artery (fig. 1). It was firmly attached to the ligament tissues that were in front and on the right side of the spine opposite the first to fourth lumbar vertebrae. At the upper edge it had invaded the psoas muscle, but lower down the muscle tissues were displaced laterally and anteriorly. The right kidney was separate. Surfaces made by cutting the mass were mainly tan brown, hemorrhagic, leathery and fibrous tissues. No enlarged iliac lymph nodes were on either side. As indicated in the anatomic diagnosis, there were many metastases in numerous parts of the body, in the lungs especially. The body of the right testis was 4 by 2.5 by 2.7 cm. The tunics were smooth; surfaces made by cutting were brown, and threadlike tubules could be pulled out. The epididymis was small and had no tumor.

The right retroperitoneal tumor and many metastases were examined histologically. All had trophoblastic Langhans and syncytial cells, associated with extensive hemorrhages (fig. 2). The right testis had atropic or hypoplastic germinal cells without a trace of spermatogenesis. The interstitial cells were abundant and apparently increased in amount.

COMMENT

The occurrence of cancerous trophoblastic tissues in the testis and other (extragenital) male germinal tissues is accepted. Many observers discussing the origin of testicular teratomas have hypothesized a parthenogenetic or a hermaphroditic process. Fortner and Owen [11] apparently regarded an ovum component as an essential factor in the growth of a choriocarcinoma but doubted that the male gland possessed structures which had the morphologic value of an ovum and which, in turn, had trophoblastic tissues. Petillo,[12] in 1944, reviewed the theories concerning the pathogenesis of teratomas and choriocarcinomas of the testis. The latter, he said, should be termed embryochoriomas and not teratomas. He proposed the hypothesis that a sex reversal may occur in the human gonads which makes possible an actual fusion of ova and spermatozoa. This autofertilization initiates a growth of one or more

11. Fortner, H. C., and Owen, S. E.: Am. J. Cancer **25**:89, 1935.

12. Petillo, D.: Urol. & Cutan. Rev. **48**:53, 1944.

embryos which die, leaving as survivals the chorion and occasional vestiges of other tissues. He did not state how these tissues then become cancerous instead of remaining local and limited in growth.

Petillo explained the great disparity of the testicular and the ovarian occurrence of these tumors on the basis that there is a greater opportunity for sex reversal in the testis because most oogonia have advanced to genetic maturity before puberty. Therefore, ovarian sex reversal is possible only in the first decade of life and accordingly choriocarcinoma of the ovary is a disease of childhood.

Petillo's parthenogenetic origin of teratomas of the testes proposes (1) a highly theoretic sex reversal of germinal tissues of a male which occurs long after the testes have reached functional development, (2) the maturation of this sex reversed element, a potential ovum, and (3) the fertilization of this female element by a sperm. Apparently in many theories of new growths of germinal tissues, including the choriocarcinomas, the reasoning is dominated by the idea that a process of fertilization initiates the growth. Present views of the origin of cancerous tumors of other tissues of the body do not hypothesize a union of such maturated cell elements, a fertilization process, as the initiating phase of the growth. Choriocarcinoma arising in women with pregnancy is a cancerous growth of trophoblastic tissues of the placenta, a structure adventitious to the fetus but having its origin in the mass of cells resulting from fusion of an ovum and a sperm. So long as this was the only way in which these cancerous growths were known to occur, an actual fusion of the male and female elements seemed essential. Now, because choriocarcinoma is known to occur often in testes, rarely in extragenital tissues (probably germinal) in males, and in the ovaries of only a few female children or adolescents, the fertilization process as such has less significance. Trophoblastic tissues, the characteristic element of these cancerous tumors, may come from potential elements in either the sperm or the ovum, and not necessarily from the composite cells arising from both. According to this reasoning, in the origin of choriocarcinoma complicating a pregnancy, fertilization provides only some of the conditions favorable for the development of this cancerous tumor. Friedman and Moore [7] have stated that embryonal carcinomas and teratoid tumors, which consist of evolving and differentiating somatic and trophoblastic tissues, are neoplastic expressions of embryonic cells.

In the male, choriocarcinomas arise in germinal tissues. According to Symeonidis, only teratomas arising in male germinal or pregerminal cells have the ability to produce trophoblastic tissues. Further, choriocarcinomas, whether they lie within the germ tissues or elsewhere (extragenital), he stated, are derivatives of germinal cells, so far testicular.

Accordingly, certain comments seem proper in considering the entire incidence of choriocarcinomas. In the adult female the tumor occurs in

the uterus or adnexa as a complication of pregnancy, that is, through fertilization of an ovum by which a male and a female sex element are fused. In the adult male it occurs just as exclusively in the testis or in apparent displacements of testicular tissues. The mature testis, according to the information now available concerning its structure, has no maturated ova. The conclusion seems to follow that trophoblastic tissues are a characteristic of the male germinal tissues (testis), not of the mature female (ovary), and that the male element contributes the trophoblastic tissue component when chorioçarcinoma develops from a fertilized ovum in the uterus or adnexa as a complication of pregnancy. The almost total absence of reports of choriocarcinoma of the ovary of the adult female supports this view. As Symeonidis has suggested, perhaps the trophoblast of the fertilized ovum forms because of influences introduced by the male component. Stated in a more direct way, the testicular choriocarcinomas seem to provide evidence that trophoblastic tissues are contributed by the male component in the normal development of a fertilized ovum in the uterus.

SUMMARY

Choriocarcinoma, originally regarded as a cancer of the trophoblastic tissues of the placenta, occurs also, in many instances, in the testis and, in a few instances, in the ovary of the young or adolescent females and, in a small number of cases, in extragenital tissues, usually retroperitoneal or mediastinal, but in these cases exclusively in the male.

Chorionic gonadotropin, the active principle of the Aschheim-Zondek reaction, appears in the urine of patients with choriocarcinoma and provides biologic evidence, in addition to the tissue structure, that the tumor contains trophoblastic elements.

Many cases of choriocarcinoma have been described among cases of cancer of the testis of the fully mature male; only a few cases of choriocarcinoma of the ovary of the young or adolescent female have been reported. In the recorded instances of extragenital choriocarcinoma the tumor was observed only in fully mature males.

The process of fertilization, parthenogenetic or hermaphroditic, has seemed convenient to use theoretically for the explanation of these tumors, probably, at first, because choriocarcinoma was recognized as a cancerous complication of pregnancy. Trophoblastic tissues theoretically may be derived from potential elements of either the sperm or the ovum, not necessarily from the composite tissues produced by the fusion of the two. Fertilization, accordingly, simply provides conditions favorable for the growth of choriocarcinoma.

Tumors of this type, exclusive of those complicating pregnancy in fertile women and a remarkably small number encountered in ovaries of

immature or adolescent females, which may be actually testicular, occur in large numbers in testes of mature males and in small numbers in extragenital tissues considered to be germinal, also in mature males.

The conclusion seems to follow that trophoblastic tissues are a characteristic of mature male germinal tissues (testis), not of the mature female (ovary), and that the male element contributes the trophoblastic tissue component when choriocarcinoma develops in the uterus or adnexa as a complication of pregnancy. This could also imply that the testicular choriocarcinoma provides evidence that the trophoblastic tissues (placenta) are contributed by the male element in the normal development of a fertilized ovum in the uterus.

Another case of extragenital, retroperitoneal choriocarcinoma, in which the patient was an adult male, is recorded.

ACUTE CLOSED CEREBRAL LESIONS TREATED BY INJECTION OF HYPERTONIC DEXTROSE SOLUTION AND BY SURGICAL DECOMPRESSION

A Quantitative Study

C. B. TAYLOR, M.D.
GEORGE M. HASS, M.D.
AND
JOHN E. MALONEY, M.D.
CHICAGO

PREVIOUS studies have shown that acute closed cerebral lesions can be produced hypothermally in rabbits without interrupting the continuity of the calvarium or introducing variables incidental to mechanical trauma.[1] The lesions were controlled as to dimensions and locations so that they could be reproduced topographically and quantitatively in successive animals. Although hemorrhage, edema and necrosis varied slightly in lesions which were otherwise identical, the variations were restricted to the discrete volumes of cerebral injury. Animals rarely showed symptoms when unilateral or bilateral cerebral lesions occupied less than 9.4 volumes per cent of brain. Severe symptoms leading to death often developed when lesions occupied 9.4 to 18.5 volumes per cent of the brain. When lesions were within this range of magnitude, the average minimum lethal volume of cerebral damage in 50 per cent of the animals ($M.L.V._{50}$) was 14.3 volumes per cent of the brain. Several clinical courses with an average postoperative duration of about seven hours and fatal termination always occurred when lesions occupied more than 18.5 volumes per cent of the brain. The great majority of animals that died had a postoperative period of normal behavior lasting one to six hours. This was followed by a secondary lapse into stupor which was a clear indication of impending coma and eventual death, the animal succumbing within at least twenty-four hours from the time of completion of the operation.

These data indicated that the method might be useful for a study of the rate of onset and decline of factors responsible for death. Multiple

This research was aided by a grant from the Otho S. A. Sprague Memorial Institute Fund.

From the Rush Department of Pathology, Presbyterian Hospital, in affiliation with the Department of Pathology, University of Illinois College of Medicine.

1. Taylor, C. B.; Hass, G. M., and Maloney, J. E.: Arch. Path. **47**:450, 1949.

sublethal lesions were made, successively, in animals at twenty-four, forty-eight, seventy-two and ninety-six hour intervals.[2] These studies showed that the factors responsible for death began to subside after twenty-four hours had elapsed and by the end of forty-eight hours had largely disappeared. It was suggested that cerebral congestion and edema were the most important of these factors.

These observations established methods and criteria for a quantitative study of the treatment of acute closed cerebral lesions characterized by local edema, hemorrhage and necrosis. It seemed that any treatment, to be effective, would have to be instituted early and that the effectiveness of the treatment could be measured, either in terms of survival of animals with lesions of lethal magnitude or in terms of survival of animals with symptoms indicating the presence of a lethal lesion. Furthermore, it seemed that the degree of effectiveness of the treatment might be evaluated, quantitatively, in terms of dimensions of lesions. With these points in mind a study was planned for the purpose of inquiring into the merits of intravenous injection of hypertonic dextrose solution and surgical decompression as methods of treatment.

METHODS

Albino rabbits, 3 to 6 months of age and weighing 4 to 6 pounds (1,814 to 3,721 Gm.), were used. Closed intracerebral lesions were produced by a method described elsewhere.[1] This method consisted essentially of aseptic exposure of the calvarium of the vertex of the skull followed by freezing of the calvarium and underlying cerebrum with a special instrument. One or more simultaneous lesions were produced in one or both cerebral hemispheres, care being taken to produce a lethal or near lethal volume of cerebral injury. The range of the quantity of damage was 8.9 to 51.2 volumes per hundred volumes of the brain. The scalp was then closed with silk, and treatment was instituted at various times in the postoperative period.

Twenty-four animals were treated by intravenous administration of 10 or 25 per cent aqueous solutions of dextrose. (See table 1.) The dextrose was given in a vein of the ear, with the head and neck of the animal immobilized by an adjustable bivalved plaster cast. Under these conditions the animal ordinarily lay quietly at rest throughout the period of administration of fluid. The rate of administration of dextrose solutions was controlled by a mercury drop displacement method. The rate varied from as much as 50 cc. per minute, when separate intravenous injections were given, to 6 to 8 cc. per hour when intravenous injections were continuous.

Separate injections of 10 per cent dextrose solution were given intravenously as follows: Each of 6 animals was given a single injection immediately after the operation; 1 was given 17 cc. and 5 received 50 cc. Each of 2 animals was given a single injection at the end of the first postoperative hour; 1 was given 17 cc. and 1, 50 cc. Three animals received total amounts of 45, 50 and 55 cc. of dextrose solution, respectively, by two or more injections at short intervals of time in the immediate postoperative period.

2. Taylor, C. B.; Hass, G. M., and Maloney, J. E.: Arch. Path. **48**:195, 1949.

Continuous administration of 25 per cent dextrose solution, 6 to 8 cc. per hour, the total amounts varying from 4 to 182 cc., was used in 13 animals. The injection was continuous for twenty-four hours if the animal survived. At the end of twenty-four hours the injection was stopped, because previous experience had shown that if an animal survived for twenty-four hours it recovered completely, except for rare instances in which an animal lived for days in a lethargic, semistuporous state.

TABLE 1.—*Relations Between Percentage Volume of Cerebral Damage and Survival of Animals Treated by Intravenous Injection of Dextrose*

	Intravenous Therapy After Production of Lesions			
Percentage Volume of Cerebral Damage *	Method of Postoperative Intravenous Administration of Dextrose	Dextrose Concentration, Percentage	Total Amount of Solution Used, Cc.	Duration of Life After Production of Lesions, Hr.
8.9	Continuous infusion	25	177	23
11.0	Continuous infusion	25	30	4
12.1	Immediate single infusion	10	50	Survived
12.6	Continuous infusion	25	178	Survived
12.8	Delayed single infusion 1½ hours postoperatively	10	50	3
13.3	Continuous infusion	25	151	Survived
14.5	Immediate single infusion	10	17	Survived
14.6	Immediate single infusion	10	50	10
14.7	Two infusions at 0 and 1 hour postoperatively	25	50	2
14.9	Continuous infusion	25	12	2.25
16.3	Two infusions at ½ and 1 hour	10	45	6
16.7	Delayed single infusion 1 hour postoperatively	10	17	3.75
16.9	Immediate single infusion	10	50	Survived
17.5	Continuous infusion	25	168	Survived
18.0	Continuous infusion	25	64	15
18.4	Continuous infusion	25	44	8
18.6	Four infusions at 0, 1, 2, 3 hours postoperatively	10	55	4
19.3	Immediate single infusion	10	50	6.5
19.5	Continuous infusion	25	182	Survived
19.6	Continuous infusion	25	20	2.75
19.7	Continuous infusion	25	12	1.25
21.2	Immediate single infusion	10	50	2.25
24.7	Continuous infusion	25	5	0.75
30.0	Continuous infusion	25	4	0.75

* The percentage volume of cerebral damage represents the number of cubic millimeters of lesion per hundred cubic millimeters of brain.

Three rabbits in which closed cerebral lesions had not been produced were given 25 per cent dextrose solution, 6 to 8 cc. per hour, intravenously for twelve, twenty-four and thirty-six hours. This was done to determine the effect of this type of treatment on normal animals.

Surgical decompression was done by excision of a circular, quadrangular or elliptic piece of the calvarium. The bone was cut by a thin rotating emery disk attached to the terminal fitting of a flexible cable powered by an electric motor (a dental grinding and polishing instrument). The emery disk quickly cut through the bone at the periphery of the bone flap. The bone flap was then elevated and separated from the dura. Occasionally, a small cut was inadvertently made in the dura while the bone flap was being removed. In general, however, trauma of the brain was minor, and escape of cerebrospinal fluid was negligible

TABLE 2.—*Relations Between Percentage Volume of Cerebral Damage and Survival of Animals Treated by Surgical Decompression Over Each Lesion Thirty to Forty-Five Minutes After Production of Lesion*

Dimensions of Parietal Lesions				Dimensions of Decompressions		Percentage Volume of Cerebral Damage	Duration of Life After Production of Lesions
Right		Left					
Surface Area, Mm.²	Depth, Mm.	Surface Area, Mm.²	Depth, Mm.	Right Area, Mm.²	Left Area, Mm.²		
154	4.0	...	...	105	...	9.5	Survived
243	5.0	...	...	142	...	10.0	Survived
228	5.0	...	...	149	...	11.0	Survived
149	4.5	170	5.0	61	100	12.5	Survived
351	5.0	...	...	138	...	12.6	Survived
290	4.5	...	...	150	...	13.1	Survived
325	5.0	...	...	141	...	13.3	Survived
355	5.0	...	...	125	...	13.5	Survived
342	5.5	...	...	143	...	14.0	Survived
327	5.0	...	...	123	...	14.2	Survived
385	5.0	...	...	223	...	14.6	16 hours
328	4.0	71	4.0	160	...	14.5	10 hours
387	5.0	...	...	142	...	14.9	Survived
390	5.0	...	...	132	...	15.0	Survived
330	5.0	...	...	141	...	15.0	7 hours
434	5.0	...	...	218	...	15.4	4 hours
362	5.0	...	...	118	...	15.6	Survived
407	5.0	...	...	142	...	15.7	Survived
410	5.0	...	...	181	...	16.4	Survived
343	6.0	...	...	198	...	16.5	Survived
369	6.0	...	...	154	...	16.6	14 hours
362	3.0	324	3.5	94	110	17.6	4 hours
54	4.5	432	4.5	...	109	17.8	7 hours
281	5.0	222	4.5	41	78	18.3	Survived
427	7.0	...	...	135	...	19.0	Survived
437	5.5	...	...	110	...	20.0	9 hours
312	4.0	324	4.0	88	91	20.4	5 hours
542	5.5	...	...	160	...	20.6	Survived
430	6.0	...	...	138	...	21.2	9 hours
448	6.0	...	...	120	...	21.3	Survived
331	5.0	314	3.0	92	100	22.4	13 hours
461	6.5	...	...	144	...	23.4	Survived
306	5.0	329	4.0	94	85	23.5	Survived
324	5.0	309	5.0	56	52	24.0	7 hours
294	4.0	285	6.0	65	47	24.0	Survived
338	5.0	318	4.5	105	128	26.0	18 hours
321	6.0	324	5.5	131	94	26.5	Survived
439	3.5	420	5.0	83	75	27.7	7 hours
388	5.0	360	4.5	150	95	28.5	3 hours
305	4.2	315	4.3	84	94	29.2	16 hours
416	4.0	420	4.0	80	89	29.3	3 hours
490	3.5	408	4.2	118	101	29.6	4 hours
360	4.5	358	5.0	107	99	29.9	Survived
350	5.5	350	5.5	112	118	32.3	Survived
310	7.0	250	7.0	119	125	32.7	Survived
375	6.0	393	6.0	92	101	33.5	3 hours
458	7.5	403	7.8	129	104	51.2	4 hours

because the swollen cerebral cortex herniated into the linear dural defect. The scalp was then closed with silk.

Three groups of animals were treated by surgical decompression with removal of bone flaps. Group 1 consisted of 47 animals with unilateral or bilateral lesions.

(See table 2.) Surgical decompression was done within thirty to forty-five minutes after production of the lesions. Bone flaps were made over the lesions. The area of bone removed over each lesion was usually one-fourth to one-half the area of the cortical surface of the lesion. (See table 2.) Group 2 consisted of 4 animals with unilateral lesions. (See table 3.) Surgical decompression was done within thirty to one hundred and five minutes after the production of lesions. Bone flaps were made over the cerebral hemisphere contralateral to the location of the lesion. The area of bone removed over the normal hemisphere was about one-half the area of the surface of the cortical lesion in the opposite hemisphere. Group 3 consisted of 14 animals with unilateral or bilateral lesions. Surgical

TABLE 3.—*Relations Between Percentage Volume of Cerebral Damage and Survival of Animals Treated by Surgical Decompression*

Percentage Volume of Cerebral Damage	Hours Between Production of Lesions and Onset of Symptoms		Hours Between Production of Lesions and Surgical Decompression		Duration of Life After Production of Lesions, Hr.
	Stupor	Convulsions	Over Lesions	Contralateral to Lesions	
11.7	...	....	....	1.50	Survived
12.6	...	....	....	6.50	4.00
13.5	5.0	....	....	1.75	5.25
15.2	5.0	....	....	1.50	5.50
12.3	...	1.5	....	....	1.50
12.4	...	....	....	....	Survived
14.8	...	1.5	....	....	1.50
17.0	2.0	2.75	....	....	2.75
19.1	...	1.00	....	....	1.00
19.2	4.0	....	4.0	....	Survived
19.4	...	3.50	3.50	....	Survived
19.6	4.0	....	4.00	....	Survived
20.5	...	1.00	....	....	1.00
21.0	1.5	....	1.50	....	1.75
21.5	6.0	....	6.00	....	Survived
22.2	...	1.00	1.00	....	Survived
24.7	1.5	....	1.75	....	Survived
49.4	...	0.75	1.00	....	2.25

decompression was delayed until the postoperative onset of stupor or convulsions, symptoms regularly indicative of a fatal prognosis in untreated animals. These symptoms developed within one to six hours after the production of lesions. In this group the decompression had to be done rapidly, because in untreated animals death often promptly followed the onset of symptoms. It was possible to do a decompression in only 8 of the 14 animals. Five died before the operation could be undertaken. One survived without symptoms. (See table 3.) Decompressions were done over the lesions. Each flap of bone removed had a surface area equal to about one-fourth to one-half the surface area of the subjacent cortical lesion.

Postmortem studies were made in all instances. Animals which survived were killed at the end of twenty-four hours. The volume per cent of cerebral damage was determined by methods described elsewhere.[1] In rabbits it represents the number of cubic millimeters of lesion per hundred cubic millimeters of brain. By study of these three groups of animals, we were able to compare, first, the relative merits of homolateral and contralateral decompression and, second, the relative merits of immediate and delayed homolateral decompression.

RESULTS

Six animals were given a single intravenous injection of a 10 per cent aqueous solution of dextrose immediately after the production of cerebral lesions. (See table 1.) Three lived and 3 died. In those that lived the cerebral damage totaled 12.1, 14.5 and 16.9 volumes per hundred volumes of the brain. In those that died the cerebral damage totaled 14.6, 19.3 and 21.2 volumes per cent of the brain. The M.L.V.$_{50}$ was 14.5 volumes per cent. This was comparable with 14.3 volumes per cent in a previously reported untreated control series.[1] The maximum

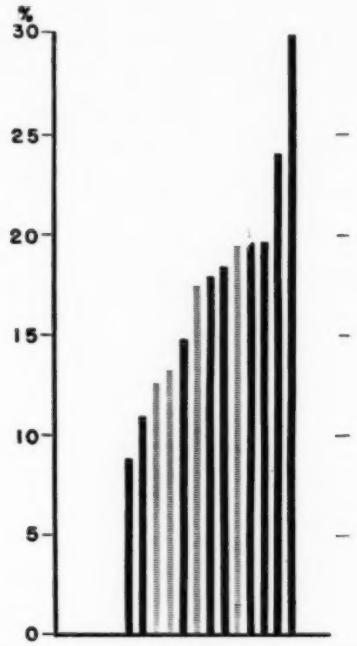


Chart 1.—Each bar represents a rabbit given an intravenous injection of a 25 per cent aqueous solution of dextrose, continuously, after production of the percentage volume of cerebral damage indicated by the height of the bar. The interrupted bars represent animals which survived and the solid bars animals which died.

quantity of survivable cerebral damage was 16.9 volumes per cent. This was comparable with 18.0 volumes per cent in a previously reported control series.[1] It seemed that treatment of this type had little or no promise; so the experiment was terminated.

Two animals were given a single intravenous injection of a 10 per cent aqueous solution of dextrose a short time after the production of cerebral lesions. (See table 1.) They died with cerebral damage of 12.8 and 16.8 volumes per cent,

respectively. As indicated by data discussed in the preceding paragraph, this method showed little promise of benefit and was dispensed with.

Three animals were given a combination of immediate and delayed postoperative intravenous injections of 10 per cent dextrose. (See table 1.) All died with cerebral damage of 14.7, 16.3 and 18.6 volumes per cent, respectively. As indicated before, the results showed that the treatment had little promise, and it was not studied further.

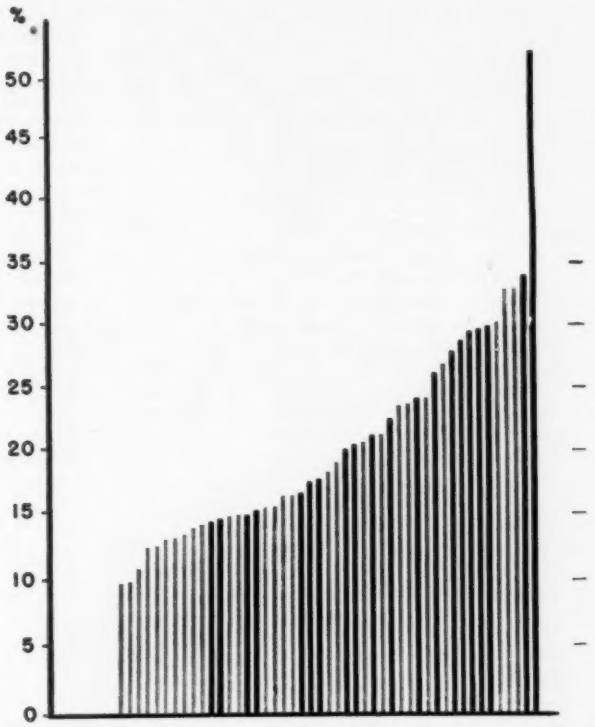


Chart 2.—Each bar represents a rabbit whose brain was surgically decompressed within thirty to forty-five minutes after production of the percentage volume of cerebral damage indicated by the height of the bar. The interrupted bars represent animals which lived and the solid bars animals which died.

Thirteen animals were given continuous intravenous injections of 25 per cent dextrose. (See table 1.) The administration was started immediately after the production of cerebral lesions and continued at the rate of 6 to 8 cc. per hour until the animal died or until complete recovery was apparent at the end of twenty-four hours. Nine animals died. Four survived. Those which died had cerebral damage of 8.9, 11.0, 18.0, 18.4, 19.6, 19.7, 24.7 and 30.0 volumes per cent, respectively. In those which survived the cerebral damage totaled 12.6, 13.3, 17.5

and 19.5 volumes per cent, respectively. Calculations indicated that the M.L.V.$_{50}$ was about 14.5 volumes per cent of the brain. (See chart 1.) The maximum survivable quantity of cerebral damage was 19.5 volumes per cent of the brain. Comparison of these data with those given previously for the control series of untreated animals showed that this treatment was of no significant value. It seemed possible that too much dextrose might have been given in some instances, but similar treatment of normal animals, not operated on, had no adverse effect. Hence, further plans to seek an effective means of getting a beneficial result with hypertonic solution of dextrose were given up.

Surgical decompression with the bone flap being lifted on the side opposite the location of the cerebral lesion was done in 4 animals. (See table 3.) Three died with cerebral damage of 12.6, 13.5 and 15.2 volumes per cent, respectively. One lived with cerebral damage of 11.7 volumes per cent. These data indicated that the operation was of no benefit and seemed to decrease the chance of survival. Hence, no further operations of this type were done.

Surgical decompression with the bone flap being made over each lesion within thirty to forty-five minutes after production of the lesions was done in 47 animals. (See table 2.) Some animals had unilateral and some bilateral bone flaps. As a rule, the area of decompression was one-fourth to one-half the surface area of the subjacent cortical lesion. Smaller decompressions were relatively ineffective and were dispensed with in early experiments. Larger decompressions were difficult to do, technically. The quantity of cerebral damage varied from 9.8 to 51.2 volumes per cent of the brain. All animals with less than 14.6 volumes per cent survived. This value was considerably greater than the 9.4 volumes per cent of a previously reported untreated control series.[1] The maximum survival quantity of cerebral damage was 32.7 volumes per cent of the brain. This was also much greater than the 18.0 volumes per cent in a previously reported untreated series. There were 35 animals with a quantity of cerebral damage in the range 14.6 to 32.7 volumes per cent of the brain; 17 survived and 18 died. The M.L.V.$_{50}$ was about 21.9 volumes per cent of the brain. (See chart 2.) This was about 50 per cent greater than the M.L.V.$_{50}$ (14.3 volumes per cent) of the previously reported untreated control series.[1]

Surgical decompression was delayed in 8 animals until the onset of stupor or convulsions. (See table 3.) These symptoms, indicative of a fatal lesion in untreated rabbits, developed within forty-five minutes to six hours after production of lesions. Decompressions were then done by removing bone flaps over the lesions. Six animals survived with cerebral damage of 19.2, 19.4, 19.6, 21.5, 22.2 and 24.7 volumes per cent, respectively. One animal died with 21.0 volumes per cent and another with 49.4 volumes per cent. The average quantity of cerebral damage among the 6 surviving animals was 21.1 volumes per cent of the brain. This beneficial result was of about the same magnitude as that achieved by immediate decompression over the lesions.

COMMENT

Previous studies have shown that discrete cerebral lesions characterized by acute necrosis, hemorrhage and edema can be reproduced, topographically and quantitatively, in successive animals without opening the skull or employing methods which subject the brain to mechanical trauma.[1] It was shown that in rabbits the average minimum lethal quantity of cerebral damage was 14.3 per cent of the brain by volume.

No animal survived with a quantity of damage in excess of 18.0 per cent. It was observed that most animals which died had a period of normal postoperative behavior. Usually, this lasted one to six hours and was followed by stupor and coma, terminated by convulsions and death within twenty-four hours after the production of lesions. Further studies disclosed that the factors responsible for death continued to exert a progressive effect, if the animal survived, until about twenty-four hours postoperatively.[3] After this time the influence of the factors subsided rapidly, so that at the end of forty-eight hours it was possible to produce a second acute lesion of nearly lethal dimensions with survival of the animal. The evidence indicated that cerebral congestion and edema in and around the lesion were the most important factors.

There were reasons for believing that the experimental situation created by these lesions was a fair anatomic reduplication of the situation which exists following some vascular accidents occurring in the human cerebrum or in intracerebral tumors.[4] In patients the acute onset of symptoms and the frequent rapid progression of severe symptoms lead often to a critical condition which may be treated in a variety of ways. If the condition is secondary to an intracerebral vascular accident, usually in an elderly person, either nothing is done or some form of dehydration therapy, such as the intravenous injection of a hypertonic solution of dextrose, is employed. If the condition is secondary to trauma of the head, dehydration therapy with a course of watchful waiting is the usual method of treatment. There is little sound experimental basis for divergence of views as to a proper method of treatment in any instance. The prevailing methods have largely been developed through experience. The difficulty lies in comparing, quantitatively and topographically, the lesions in one case with those in another. Views which are clearly defended by adequate data are difficult to find.

For these reasons the present experiments were undertaken. The results showed that in dealing quantitatively with reproducible acute closed cerebral lesions the intravenous injection of a hypertonic solution of dextrose, whether continuous or intermittent, was of no benefit to the animal from the point of view of either amelioration of symptoms or survival. These data do not deny the utility of dextrose as a source of nutrition. They simply cast doubt on the utility of intravenous injections of hypertonic solutions of dextrose as a means of combating the intracranial factors which lead to severe cerebral symptoms and death from cerebral causes.

On the other hand, the data showed that surgical decompression was of benefit if certain rules were followed in doing the decompression. First, decompression was of little or no benefit unless it was done over the site of the lesion. Second, the area of bone removed had to be at least one-fourth to one-half the submeningeal surface area of the lesion

to obtain a satisfactory benefit. Third, although decompression done shortly after the production of lesions was more beneficial than delayed decompression, the difference was not significant. When the rules suggested were followed, severe cerebral symptoms were ameliorated in many animals and the average amount of cerebral damage which could be tolerated with survival was increased about 50 per cent. (See chart 3.)

In view of these findings it may be worth while to reevaluate surgical decompression as a method of treating selected patients for intracerebral vascular accidents. The prospective use of this method of treatment should be guided largely by the ability of the clinician to make an

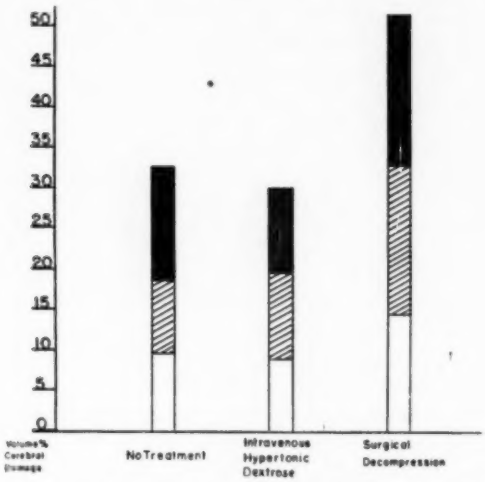


Chart 3.—This graph compares experience gained in a study of three groups of rabbits with acute cerebral damage. One group, previously reported, was a large untreated series.[1] The group treated by intravenous injection of dextrose solution and that by surgical decompression over the sites of the cerebral lesions as described in this paper are represented in the second and third bars. In these bars the lethal range of cerebral damage is shown by solid black (no survival); the survivable range by stripping (41, 63 and 51 per cent mortality, respectively); the survival range, by solid white (no mortality).

accurate anatomicopathologic diagnosis and prognosis before considering decompression as a life-saving measure.

SUMMARY

Acute closed cerebral lesions characterized by hemorrhage, necrosis and edema were produced in rabbits with a hypothermal instrument placed against the external table of the calvarium. Following the pro-

duction of lesions, some animals were treated by intravenous injection of either 10 or 25 per cent aqueous solutions of dextrose and some by surgical decompression. There was no evidence that hypertonic solutions of dextrose given periodically or continuously by the intravenous route had any influence on the clinical course or the mortality rate. There was good evidence that surgical decompression was of great benefit if certain rules were followed. Removing a bone flap on the side opposite the site of the lesion was of no benefit. Removing a bone flap over the site of the lesion was of definite benefit, so that it was possible to save lives of animals, even after onset of symptoms indicating impending death, with a volume of cerebral damage averaging 50 per cent more than the average volume tolerated without treatment. The data indicated that surgical decompression should be reevaluated as a method of treatment for patients with certain types of intracerebral vascular accidents.

EFFECTS OF PROLONGED ADMINISTRATION OF ANTITHYROID COMPOUNDS ON THE THYROID AND OTHER ENDOCRINE ORGANS OF THE RAT

JOSEPH SEIFTER, M.D.
WILLIAM E. EHRICH, M.D.
AND
GEORGE M. HUDYMA, B.S.
PHILADELPHIA

THE IMMEDIATE effects of antithyroid drugs are well known, but the changes produced in the thyroid gland and other tissues by prolonged administration of these compounds have not been adequately described.

Rats fed a rape seed diet for ten months or longer revealed benign adenomas in the thyroid gland,[1] while rats given 0.25 per cent thiourea in their drinking water for twenty months or longer showed carcinomas.[2] Female mongrel dogs receiving thiouracil for six months from the time of weaning had undeveloped gonads and accessory sex organs, abnormal dentition, and delayed growth and epiphysial closure of the long bones[3]; after a detailed study of the teeth and skulls of these dogs, English[4] presented evidence of adaptation to the effects of thiouracil. Cats responded atypically with atrophy of the thyroid gland, changes in the male gonads and necrosis of the adrenal cortex.[5]

During a study of the effects of prolonged administration of antithyroid compounds in rats, we encountered changes in the thyroid gland and the anterior lobe of the pituitary gland not heretofore reported. We also observed adenoma and carcinoma in the anterior lobe of the pituitary gland, the adrenal glands and elsewhere.

MATERIALS AND METHODS

The antithyroid compounds were administered for fourteen weeks to two years in the standard laboratory diet (Purina® dog chow) of white rats from weaning

From the Wyeth Institute of Applied Biochemistry, Wyeth Incorporated, and the University of Pennsylvania, Graduate School of Medicine.

1. Griesbach, W. E.; Kennedy, T. H., and Purves, H. D.: Brit. J. Exper. Path. **26**:18, 1945.
2. Purves, H. D., and Griesbach, W. E.: Brit. J. Exper. Path. **28**:46, 1947.
3. Seifter, J.: Federation Proc. **6**:370, 1947.
4. English, J. A.: J. Dent. Research **28**:172, 1949.
5. McClosky, W. T.; Lillie, R. D., and Smith, M. I.: J. Pharmacol. & Exper. Therap. **89**:125, 1947.

until death. Control groups received unadulterated standard diet. The animal quarters were air conditioned and maintained at 72 F.

The fate and the disposition of all the rats in the present study were as follows: Four rats received 0.05 per cent para-aminobenzoic acid continuously in the diet; 1 of these was killed at the end of fourteen weeks and 3 at the end of thirty-five weeks. Four rats received 0.1 per cent para-aminobenzoic acid continuously in the diet; 1 was killed at fourteen weeks, 1 died spontaneously after twenty weeks and 2 were killed at the end of thirty-five weeks. Five rats received 0.5 per cent para-aminobenzoic acid continuously in the diet; 1 was killed at the end of twelve weeks, 1 at twenty-four weeks and 3 at fifty weeks.

Four rats received 0.001 per cent thiouracil continuously in the diet; 1 was killed at the end of fourteen weeks, and 3 were killed at thirty-five weeks. Twelve rats received 0.005 per cent thiouracil in the diet; 5 died spontaneously, 1 at five weeks, 1 at thirty-five weeks, 1 at fifty-eight weeks, 1 at seventy-six weeks and 1 at one hundred and three weeks; 7 were killed, 1 at five weeks, 1 at fourteen weeks, 2 at thirty-five weeks, 2 at fifty-two weeks and 1 at one hundred and four weeks. Eight rats received 0.01 per cent thiouracil in the diet; 4 died spontaneously, 1 at eighty-seven weeks, 1 at eighty-nine weeks and 2 at ninety-two weeks; 4 were killed, 2 at fifty-two weeks, 1 at eighty weeks and 1 at one hundred and four weeks.

Four rats received 0.001 per cent thiouracil plus 0.05 per cent para-aminobenzoic acid continuously in the diet; 1 was killed at the end of fourteen weeks, and 3 were killed at the end of thirty-five weeks. Four rats received 0.005 per cent thiouracil plus 0.1 per cent para-aminobenzoic acid; 1 was killed at the end of fourteen weeks, and 3 were killed at the end of thirty-five weeks. The acid was included in order to boost the goitrogenic effect without increasing toxicity. Antithyroid action,[6] low toxicity[7] and detoxifying effect[8] for certain metallo-organic compounds have been reported for para-aminobenzoic acid.

Four rats received 0.001 per cent 2-aminothiazole continuously in the diet; 3 were killed, 1 at the end of fourteen weeks and 2 at thirty-five weeks; 1 was missing at the end of twenty-five weeks. Four rats received 0.005 per cent 2-aminothiazole in the diet; 4 were killed, 1 at the end of fourteen weeks and 3 at thirty-five weeks.

Four rats received 0.001 per cent 2-aminothiazole plus 0.05 per cent para-aminobenzoic acid continuously in the diet; 4 were killed, 1 at the end of fourteen weeks and 3 at thirty-five weeks. Four rats received 0.005 per cent 2-aminothiazole plus 0.1 per cent para-aminobenzoic acid in the diet; 4 were killed, 1 at the end of fourteen weeks and 3 at thirty-five weeks. Five rats were fed 0.1 per cent 2-aminothiazole plus 0.5 per cent para-aminobenzoic acid in the diet; 1 died spontaneously at the end of six weeks, and 4 were killed, 1 at twelve weeks and 3 at fifty weeks.

Four rats received 0.001 per cent 2-aminothiazole hydrochloride continuously in the diet; 4 were killed, 1 at fourteen weeks and 3 at thirty-five weeks. Four rats received 0.005 per cent 2-aminothiazole hydrochloride; 4 were killed, 1 at fourteen weeks and 3 at thirty-five weeks.

Four rats received 0.001 per cent 2-aminothiazole hydrochloride plus 0.05 per cent para-aminobenzoic acid continuously in the diet; 1 died spontaneously at the end of five weeks, and 3 were killed, 1 at fourteen weeks and 2 at thirty-five weeks. Four rats received 0.005 per cent 2-aminothiazole hydrochloride plus 0.1 per cent para-aminobenzoic acid in the diet; 1 died spontaneously at the end of twenty-five weeks, and 3 were killed, 1 at fourteen weeks and 2 at thirty-five weeks.

6. Berman, L.: Proc. Soc. Exper. Biol. & Med. **59**:70, 1945.
7. Richards, R. K.: Federation Proc. **1**:71, 1942.
8. Sandground, J. H.: Science **97**:74, 1943.

Four rats received 0.001 per cent acetyl-2-aminothiazole continuously in the diet; 1 died spontaneously at the end of thirty-four weeks, and 3 were killed, 1 at fourteen weeks and 2 at thirty-five weeks. Four rats received 0.005 per cent acetyl-2-aminothiazole in the diet; 4 were killed, 1 at fourteen weeks and 3 at thirty-five weeks.

Four rats received 0.001 per cent acetyl-2-aminothiazole plus 0.05 per cent para-aminobenzoic acid continuously in the diet; 4 were killed, 1 at the end of fourteen weeks and 3 at thirty-five weeks. Four rats received 0.005 per cent acetyl-2-aminothiazole plus 0.01 per cent para-aminobenzoic acid in the diet; 1 died spontaneously at the end of thirty weeks, and 3 were killed, 1 at fourteen weeks and 2 at thirty-five weeks.

Eight rats received 0.0005 per cent bis-(4-acetaminophenyl) selenium dihydroxide continuously in the diet; 5 died spontaneously, 1 at seventy-five weeks, 2 at seventy-six weeks, 1 at eighty-six weeks and 1 at eighty-eight weeks; 3 were killed, 2 at fifty-two weeks and 1 at one hundred and four weeks. Eight rats received 0.005 per cent bis-(4-acetaminophenyl) selenium dihydroxide in the diet; 4 died spontaneously, 2 at sixty-six weeks, 1 at sixty-eight weeks and 1 at seventy-six weeks; 4 were killed, 1 at twenty-five weeks, 1 at twenty-seven weeks, and 2 at fifty-two weeks. Eight rats received 0.001 per cent bis-(4-acetaminophenyl) selenium dihydroxide in the diet; 6 died spontaneously, 1 at twenty-six weeks, 1 at fifty-six weeks, 1 at fifty-seven weeks, 1 at fifty-eight weeks, 1 at seventy-five weeks and 1 at ninety-five weeks; 2 were killed at the end of fifty-two weeks.

RESULTS

The amounts of antithyroid compounds administered did not materially affect the survival rate if the administration was not extended beyond thirty-five weeks. The mortality rate of rats receiving thiouracil and bis-(4-acetaminophenyl) selenium dihydroxide for one year or more was elevated 50 and 63 per cent, respectively, over the rate of 37.5 per cent of rats on the standard diet. Postmortem studies of 21 dead rats revealed pneumonia in 14, renal disease in 2 and carcinoma in 2. No cause of death was found in 3 rats.

The table lists the weights of the thyroid gland, the pituitary gland, the adrenal glands, and the thymus of each rat killed after one to two years of treatment. The thyroid glands of our untreated rats weighed less than those recorded by Donaldson.[9] The ratio of thyroid weight to body weight in the 50 Gm. rats of our series was 0.13 mg. per gram of body weight,[10] whereas in the Donaldson series it was 0.22 mg. per gram. If this difference is taken into consideration, it can be calculated from Donaldson's figures that in our animals the normal ratio should have been 0.11 mg. per gram for rats weighing 100 Gm. and 0.09 mg. per gram for those weighing 200 and 300 Gm. The table also shows that the thyroid glands of the 2 year old controls had the expected weight. One of the glands of the 1 year old controls was smaller than expected, and the other was considerably enlarged. The latter change was due to the fact that the gland was infiltrated with lymph follicles, presenting a microscopic picture resembling struma lymphomatosa of man (fig. 1*B*).

Thyroid Glands.—The thyroid glands of the rats receiving thiouracil were about three times the normal size as compared with the controls. The increase

9. Donaldson, H. H.: The Rat, Bulletin no. 6, Philadelphia, Wistar Institute of Anatomy and Biology, 1915.

10. Seifter, J., and Ehrich, W. E.: J. Pharmacol. & Exper. Therap. **92**:303, 1948.

appears to be related to the concentration of thiouracil in the diet, for it was somewhat greater with 0.01 per cent than with 0.005 per cent. The only thyroid gland that failed to enlarge to this extent was in rat 16, which had a carcinoma of the anterior lobe of the pituitary gland. In a group of 4 rats weighing 50 Gm. the thyroid gland was only twice the normal size after ten days' feeding of 0.01 per cent thiouracil in the diet.

The thyroid gland was enlarged 22 to 43 per cent in 5 of 7 rats receiving bis-(4-acetaminophenyl) selenium dihydroxide. Of the 2 with a normal size gland, rat 11 had a very small pituitary gland and rat 7 received the smallest dose of the selenium compound administered.

TABLE 1.—*Weights of Tissues of Rats Receiving Antithyroid Compounds for One and Two Years*

				Weight of							
				Thyroid Gland		Pituitary Gland		Adrenal Glands		Thymus	
	Sex	No.	Body Weight, Gm.	Mg.	Mg. per Gm.	Mg.	Mg. per Gm.	Mg.	Mg. per Gm.	Mg.	Mg. per Gm.
One Year											
Control	M	1	330	14.9	0.047	12.0	0.0375	33.8	0.106	117.2	0.366
	F	2	184	29.6	0.161	11.3	0.061	74.9	0.407	252.2	1.37
Thiouracil											
0.005%	M	3	210	57.7	0.275	18.5	0.008	33.1	0.158	171.9	0.819
	F	4	189	45.6	0.242	16.0	0.085	60.4	0.320	196.4	1.04
0.01%	M	5	283	69.8	0.247	9.1	0.032	26.9	0.095	125.6	0.444
	F	6	172	54.0	0.314	15.0	0.087	51.7	0.301	132.0	0.767
Bis-(4-acetaminophenyl) selenium dihydroxide											
0.0005%	M	7	266	24.3	0.091	17.6	0.066	37.3	0.139	153.5	0.585
	F	8	150	21.4	0.143	10.6	0.071	37.2	0.248	175.7	1.17
0.001%	M	9	294	35.1	0.119	21.1	0.072	35.2	0.119	132.0	0.449
	F	10	173	24.5	0.142	16.9	0.098	65.2	0.377	153.5	0.888
	M	11	255	19.3	0.076	9.6	0.038	50.8	0.199	110.2	0.432
	F	12	165	20.1	0.122	8.4	0.051	63.6	0.385	110.0	0.667
Two Years											
Control	M	13	339	38.2	0.113	12.5	0.037	34.6	0.102	27.5	0.081
	M	14	348	35.5	0.102	10.5	0.030	36.3	0.104	66.5	0.191
	F	15	220	18.8	0.085	24.6	0.112	93.4	0.425	100.0	0.455
Thiouracil											
0.005%	M	16	271	42.0	0.155	85.5	0.315	58.5	0.216	86.0	0.317
0.01%	M	17	254	70.1	0.276	11.2	0.044	34.2	0.135	139.1	0.548
Selenium											
0.0005%	F	18	217	24.1	0.111	32.5	0.150	125.2	0.577	101.0	0.465

All the thiouracil-treated rats showed on microscopic examination diffuse goiter involving the entire gland. Most of the follicles of rats 3, 4, 5 and 6, treated for one year, were not only considerably smaller than in the acute phase of hyperplasia but actually slightly smaller than normal (fig. 1*C*). The epithelial lining was tall, and the lumen contained little or no colloid. The degree of hyperplasia resembled that found in acute experiments with 0.01 per cent thiouracil. It was 3 plus, according to our previous grading.[10] Although most of the follicles were hyperplastic, there were single or small groups of considerably distended follicles filled with thick colloid and lined with very flat epithelium. The latter were scattered throughout the hyperplastic glands (fig. 1*C*). The thyroid gland of rat 3 also contained a few adenomas with fairly tall and deeply staining epithelium.

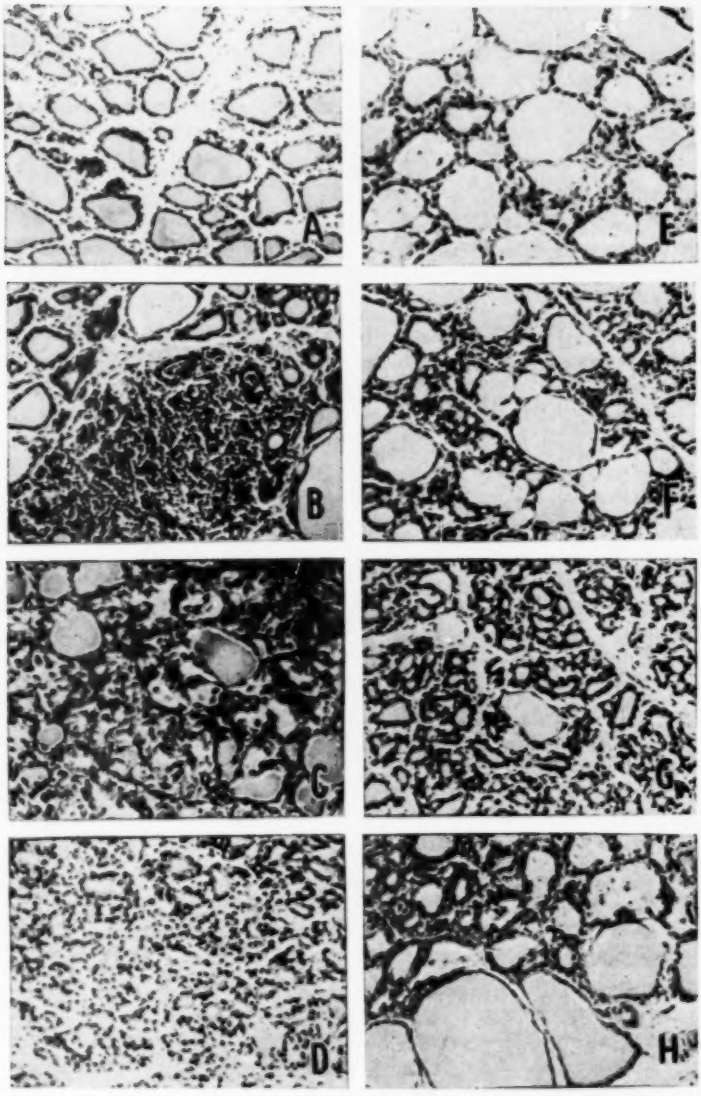


Fig. 1.—All the sections were stained with hematoxylin and eosin. The magnification was × 200. *A*, normal thyroid gland of rat 22, an untreated control. *B*, struma lymphomatosa of rat 2, an untreated control. *C*, the hyperplasia is in the diffuse hyperplasia of thyroid gland of rat 3, fed thiouracil, 0.005 per cent, for one year; transitional stage, with some colloid-containing follicles. *D*, diffuse hyperplasia microfollicular stage, in rat 17, fed thiouracil, 0.01 per cent, for two years. Note scarcity of colloid-containing follicles. *E*, macrofollicular hyperplasia (+) in rat 23, fed thiouracil, 0.001 per cent, for fourteen weeks. *F*, microfollicular hyperplasia with many colloid-containing follicles in rat 24, fed thiouracil, 0.001 per cent, for thirty-five weeks. *G*, diffuse microfollicular hyperplasia in rat 25, fed thiouracil, 0.005 per cent, for thirty-five weeks. Note scarcity of colloid-containing follicles. *H*, focal hyperplasia with preservation of peripheral follicles in rat 12, fed a selenium compound, 0.005 per cent, for one year.

The follicles of the rats killed after two years of thiouracil administration (rats 16 and 17) were minute and lined with large epithelial cells and contained little or no colloid (fig. 1*D*). These thyroid glands had no adenomas and only few distended colloid-containing follicles. They resembled the thyroid glands seen in microfollicular goiters in man, described by Smith and Gault.[11]

Administration of thiouracil for periods of less than one year also resulted in interesting changes (rats not listed in the table). The thyroid gland of a rat examined after 14 weeks of administration of 0.001 per cent thiouracil had slight hyperplasia of the usual macrofollicular type (+) (fig. 1*E*). A mixture of minute, slightly hyperplastic follicles of the microfollicular type and large colloid-containing follicles with very low epithelium appeared after thirty-five weeks of thiouracil treatment (fig. 1*F*). Increasing the concentration of thiouracil to 0.005 per cent resulted after fourteen weeks in diffuse macrofollicular hyperplasia of the thyroid gland (+ + +), including some distended follicles filled with colloid and lined with very low epithelium. Diffuse microfollicular goiter appeared after thirty-five weeks of this concentration (fig. 1*G*). The addition of para-aminobenzoic acid to the thiouracil resulted in slightly greater hyperplasia. There were no adenomas in the rats receiving thiouracil for less than one year.

The selenium-treated rats had only focal hyperplasia (+ to + +) limited to the interior of the glands, while the peripheral follicles were enlarged and filled with colloid (fig. 1*H*). There were no distended colloid-containing follicles in the interior of the glands. There were no adenomas. The follicles in the interior of the thyroid gland of rat 18, killed after two years' treatment with the selenium compound, had the identical microfollicular structure seen in the thiouracil-treated rats. In addition, however, the gland contained a solid adenoma consisting of apparently atypical thyroid epithelial cells (fig. 2*A*) with questionable mitotic figures.[12]

The thyroid glands of rats receiving 0.001 per cent of 2-aminothiazole or its derivatives for fourteen or thirty-five weeks had little or no focal hyperplasia, while all those receiving 0.005 per cent had some focal hyperplasia. Coadministration of para-aminobenzoic acid resulted in somewhat greater hyperplasia. Of 4 rats receiving 0.1 per cent 2-aminothiazole together with para-aminobenzoic acid, the one killed after fourteen weeks showed marked diffuse hyperplasia of the usual type (+ + +) but slightly greater than that observed in rats receiving only 2-aminothiazole for eleven days.[10] Of the 3 remaining rats killed after one year of administration of 2-aminothiazole and para-aminobenzoic acid, 1 showed a typical microfollicular goiter; in the other 2 the thyroid gland had well advanced changes indicative of transition to this stage. One of these (rat 19) also contained an adenoma.

Administration of 0.05 or 0.1 per cent para-aminobenzoic acid caused little or no focal hyperplasia, but 0.5 per cent produced some focal hyperplasia in all animals studied.

Pituitary Glands.—The pituitary glands of rats killed after one year's administration of antithyroid compounds were considerably enlarged except those of

11. Smith, L. W., and Gault, E. S.: Essentials of Pathology, D. Appleton-Century Company, Inc., 1938.

12. Two rats not included in this study, which died after receiving 0.075 per cent of the selenium compound for seven months, had extensive thyroid adenomas (fig. 2*B*). The mother tissue was diffusely and definitely hyperplastic, although follicles were smaller than during the acute phase of hyperplasia.

the 2 rats receiving the large dose of the selenium compound and rat 5, receiving 0.01 per cent thiouracil. The latter contained the largest thyroid gland and the smallest adrenal glands of the lot. The unenlarged pituitary glands had no spectacular microscopic changes, but 3 of the 5 enlarged glands showed a considerable increase in "degranulated" cells, i.e., in immature granulated cells or chromophobes; thyroidectomy cells were not present.

Greatly enlarged pituitary glands which showed circumscribed tumors consisting of chromophobe cells surrounded by partially compressed essentially normal tissue were seen in 2 of 3 rats killed after two years. One of these received thiouracil and the other the selenium compound. The tumor of the selenium-treated rat contained a considerable amount of iron pigment and consisted of well differentiated chromophobes showing no mitotic activity (fig. 2*C*), while the tumor of the thiouracil-treated rat revealed large atypical chromophobes with large, partly giant nuclei showing hyperchromasia and large orange-stained nucleoli, as well as abundant cytoplasm including a large Golgi apparatus (figs. 2*D* and *E*). Abundant mitotic figures were also found. Invasion was not demonstrable, but the cytologic picture could be interpreted only as indicating cancer.[13] The third rat (17) also had received 0.01 per cent thiouracil but showed no enlargement of the anterior lobe of the pituitary gland; it also contained the largest thyroid and smallest adrenal glands of the lot.

Adrenal Glands.—The adrenal glands of the thiouracil-treated rats were usually smaller than those of the controls; those of the selenium-treated rats were essentially unchanged. Enlargement of the glands was encountered in 3 rats (8, 16 and 18). That of the glands of 16 and 18 is accounted for by marked cystic degeneration of the cortex and that of rat 11 by multiple cortical adenoma. The small size of the adrenal glands of rat 8 is possibly explained by the fact that this animal was suffering from an infectious disease and that it weighed least of the lot.

The only unusual finding in the adrenal glands was that in rat 17, which had received 0.01 per cent thiouracil for two years. Both cortices had discrete tumors consisting of immature medullary cells with large nucleoli and numerous mitotic figures. The tumor cells were infiltrating the cortical tissue (fig. 2*F*). These observations suggest that these tumors were not benign adenomas but medullary carcinomas (pheochromocytomas).

Cortical adenomas were found also in 2 rats not listed in the table. One (rat 20) died in the one hundred and fourth week of receiving 0.005 per cent thiouracil, and the other (rat 21) was killed in the thirty-sixth week of receiving 2-aminothiazole. The adenoma of the latter consisted of reticularis and had mitotic figures.

Other Tissues.—The gonads of the animals did not reveal significant changes.

The thymuses of the rats receiving the highest concentrations of thiouracil or selenium for one year were smaller than those of the controls but were larger than those of the rats receiving thiouracil for two years.

Adenomas of the liver were seen in 4 rats receiving 0.0005 to 0.005 per cent bis-(4-acetaminophenyl) selenium dihydroxide for one to two years and in 1 receiving 0.005 per cent thiouracil for two years. Fibroadenomas of the breast

13. Another apparently cancerous chromophobe adenoma was found in a rat not included in this study. The animal died after seven months of a diet containing 0.075 per cent of the selenium compound and 5 parts per million of sodium arsenite.

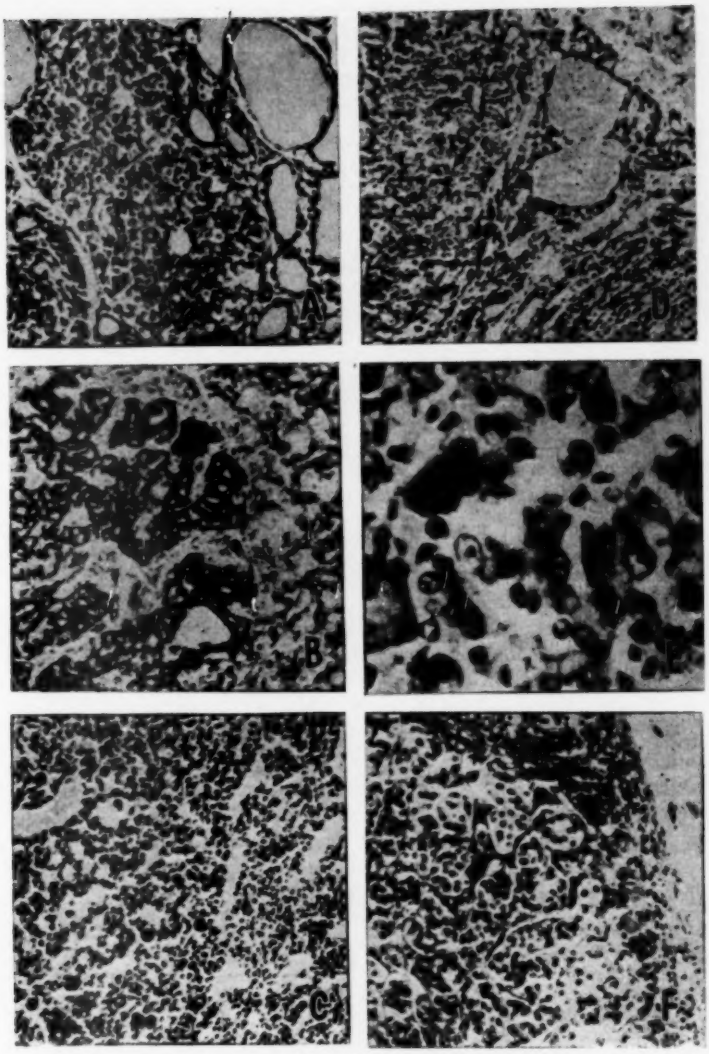


Fig. 2.—All sections were stained with hematoxylin and eosin. The magnification was × 200 except that of *E*, which was × 500. *A*, solid adenomas in thyroid gland of rat 18, fed selenium, 0.0005 per cent, for two years. *B*, simple adenoma in thyroid gland of rat 26, fed selenium, 0.075 per cent for seven months. *C*, benign chromophobe adenoma in anterior lobe of pituitary gland of rat 18, fed selenium, 0.0005 per cent, for two years. Note normal mother tissue at right. *D*, chromophobe carcinoma in anterior lobe of pituitary gland of rat 16, fed thiouracil, 0.005 per cent for two years. Note normal mother tissue at lower right. *E*, same as *D*, but magnified 500 instead of 200 times. Note the anaplasia of tumor cells. *F*, medullary adenoma infiltrating the cortex of an adrenal gland of rat 17, fed thiouracil, 0.01 per cent for two years.

were found in 1 rat fed 0.005 per cent bis-(4-acetaminophenyl) selenium dihydroxide for two years. Widespread carcinomatosis was seen in a rat that died in the sixty-seventh week of 0.005 per cent bis-(4-acetaminophenyl) selenium dihydroxide treatment.

COMMENT

It is generally believed that the thyroid gland continues to enlarge macroscopically for periods as long as fifty days.[14] In rats fed a rape seed diet, however, its growth after the twentieth day merely paralleled the body growth.[16]

From our experiments it appears that the enlargement of the thyroid gland induced by antithyroid compounds is maintained indefinitely throughout the period of administration. The only exception occured in a rat in which a chromophobe carcinoma of the anterior lobe of the pituitary gland developed. The rate of enlargement is greatest during the first few weeks of administration and gradually slows thereafter. Thiouracil when administered at a dietary level of 0.01 per cent for one to two years produced thyroid glands weighing three times as much as those of normal controls; when fed at this same level for ten days it produced glands only twice the normal size.[10] There appears to be no regression in the size of the thyroid gland after prolonged treatment.

It is also believed that the hyperplasia characteristic of the acute effects is maintained for as long as one hundred days.[14] In rats fed a rape seed diet, however, colloid reappeared in significant amounts in the follicles after the fiftieth day.[15] The fact that the "normal" thyroid tissue of a rat in which a carcinoma of the thyroid gland developed after twenty-two months of thiourea treatment was less responsive to thiourea than is usual was attributed to the cachexia and malnutrition from which the animal was suffering.[16]

In microscopic appearance the thyroid glands of the rats varied considerably. Some showed focal hyperplasia limited to the central portions; others revealed diffuse hyperplasia involving the whole gland. The minimal effective doses of the more potent antithyroid compounds and even maximal concentrations of the weakly acting para-aminobenzoic acid caused only focal hyperplasia, while larger doses of the more effective compounds caused diffuse goiters. It appears, therefore, that the intensity of antithyroid action determines whether the goiter will be diffuse or focal.

We[10] have already reported that the hyperplasia seen in acute experiments was of the usual macrofollicular type. In the present study the hyperplasia seen after prolonged administration of antithyroid

14. Charipper, H. A., and Gordon, A. S.: Vitamins & Hormones **5**:273, 1947.
15. Kennedy, T. H., and Purves, H. D.: Brit. J. Exper. Path. **22**:241, 1941.
16. Purves, H. D., and Griesbach, W. E.: Brit. J. Exper. Path. **27**:294, 1946.

compounds was of a microfollicular variety and resembled the microfollicular goiter seen in human patients by Smith and Gault.[11]

The transition to a microfollicular goiter was completed in most rats killed after thirty-five weeks of a diet containing thiouracil in concentrations of 0.005 per cent and 0.01 per cent. In 3 rats killed after one year, however, only the transitional picture from macrofollicular to microfollicular hyperplasia was seen. Similar changes were observed in rats receiving 0.1 per cent 2-aminothiazole.

The microfollicular change occurred also in glands showing focal hyperplasia. In a group of 6 rats receiving bis-(4-acetaminophenyl) selenium dihydroxide for one year, there were 4 whose thyroid glands showed transitional stages; the thyroid glands of the other 2, as well as that of a rat killed after two years of this treatment, had microfollicular goiters.

During the course of the microfollicular change the glands showing diffuse hyperplasia revealed the development of single or small groups of follicles considerably distended with thick colloid and lined with very low epithelium. These were seen as early as the fourteenth week of 0.005 per cent thiouracil but were developed best after one year of treatment; they were greatly reduced after two years of treatment. Such follicles were not observed in rats whose thyroid glands showed focal hyperplasia. Instead we found enlargement of the peripheral follicles which were not affected by the hyperplasia. These observations indicate that the distended colloid-containing follicles developing in the presence of diffuse hyperplasia are adaptation phenomena. The fact that they regress after development of the microfollicular stage suggests that the latter also is an adaptation phenomenon.

It is well established that benign and cancerous tumors of the thyroid gland develop in rats receiving antithyroid compounds for long periods.[17]

In the present study, adenomas were seen after seven months of a diet containing the selenium compound in a concentration of 0.075 per cent. One rat receiving 0.005 per cent of this compound for two years had a solid adenoma consisting of atypical thyroid epithelial cells whose benign or cancerous nature could not be established.

Studies by others have led to the conclusion that the changes in the pituitary gland coincided with those in the thyroid gland without the increase in weight. The basophilic cells rapidly increased in number and underwent hyalinization and vacuolation with the formation of "signet ring" cells, while the acidophilic cells decreased in number and underwent degranulation; the chromophobe cells were not changed.[14]

17. Dalton, A. J.; Morris, H. P., and Dubnik, C.: Federation Proc. **5**:219, 1946. Seifter, J.; Ehrich, W. E.; Hudyma, G., and Mueller, G.: Science **103**:762, 1946. Griesbach and others.[1] Purves and Griesbach.[2]

The development of "thyroidectomy cells" on a rape seed diet was maximal after fifty days and then declined so that in one hundred gland days the pituitary appeared essentially normal.[18] Rats receiving 0.1 per cent thiouracil for four months showed "fairly numerous thyroidectomy cells"; those receiving 1 per cent thiourea had "enlarged chromophobic hypophyses without acidophilic cells."[19]

In contrast to this, the pituitary glands of our rats were in most instances considerably enlarged after antithyroid compounds had been fed for one year or longer. Some of them showed no conspicuous microscopic changes, but most of the enlarged ones consisted chiefly of poorly granulated cells or chromophobes; thyroidectomy cells were no longer present. After two years of antithyroid drug treatment 2 of 3 rats showed discrete chromophobe tumors, one a benign tumor and the other a lesion having the histologic characteristics of carcinoma. The latter rat had received thiouracil at a dietary level of 0.005 per cent, and the former, bis-(4-acetaminophenyl) selenium dihydroxide at a level of 0.0005 per cent.

Our finding that adrenal glands are smaller than normal in rats treated with effective doses of thiouracil and bis-(4-acetaminophenyl) selenium dihydroxide is in agreement with the findings of Leathem [20] for acute effects of thiourea and of Leblond and Hoff [19] for chronic effects of it. Kennedy and Purves,[15] however, observed enlarged adrenal glands in rats on a rape seed diet.

Since cortical adenomas of the adrenal glands are of frequent occurrence in old rats, we attach no great significance to finding them in our animals. The presence of medullary adenomas infiltrating the cortices of a rat receiving for two years 0.01 per cent thiouracil is highly interesting, however, in view of the medullary hyperplasia observed by Bauman and Marine [21] in animals treated with thiouracil.

The failure to find changes in the gonads in the rats is in agreement with published observations,[22] as is the finding of adenomas of the liver in selenium-treated and thiouracil-treated rats.[23] The other tumors observed may have been of spontaneous rather than experimental occurrence, although a selenium effect may have been responsible, since bis-(4-acetaminophenyl) selenium dihydroxide contains 20 per cent selenium, which could possibly be released by breakdown of the compound.

---

18. Griesbach, W. E.: Brit. J. Exper. Path. **22**:245, 1941.

19. Leblond, C. P., and Hoff, W. E.: Endocrinology **35**:229, 1944.

20. Leathem, J. H.: Endocrinology **36**:98, 1945.

21. Bauman, E. J., and Marine, D.: Endocrinology **36**:400, 1945.

22. Seegar Jones, G. E.; Delfs, E., and Foote, E. E.: Endocrinology **38**:337, 1946.

23. Fitzhugh, O. G.; Nelson, A. A., and Bliss, C. I.: J. Pharmacol & Exper. Therap. **80**:289, 1944. Fitzhugh, O. G.: Science **108**:676, 1948.

SUMMARY

Rats receiving antithyroid compounds for varying periods up to two years had significant changes in the thyroid gland and, in some instances, in other endocrine glands.

The weight of the thyroid gland did not regress. Low doses of the compounds caused hyperplasia only of the central portions of the gland, while a larger intake caused diffuse hyperplasia. The macrofollicular hyperplasia characteristic of acute effects changed to microfollicular. The change was completed in some experiments as soon as the thirty-fifth week of intake of antithyroid drug. In diffuse goiters it was preceded by focal restoration of colloid. The microfollicular change and the focal restoration of colloid are interpreted as adaptation phenomena. Adenomas of the thyroid gland were also observed. They occurred earliest with administration of bis-(4-acetaminophenyl) selenium dihydroxide.

The pituitary glands of many of our rats showed hyperplasia. Thyroidectomy cells were absent after one year or later. The enlargement was due chiefly to multiplication of poorly granulated cells or chromophobes. Two rats showed chromophobe adenomas, one of which was cancerous.

The adrenal glands were atrophic in most instances. In 1 rat medullary adenomas developed in the cortices of both glands.

BILATERAL EXTENSIVE FOCAL ISCHEMIC ATROPHY OF THE KIDNEYS

HOWARD R. CHRISTOFERSEN, M.D.
AND
EDWIN F. HIRSCH, M.D.
CHICAGO

LESIONS resulting from occlusion of the branches of both renal arteries have been described in bilateral cortical necrosis of the kidneys [1] and as part of the systemic manifestations of lupus erythematosus disseminatus,[2] scleroderma,[3] rheumatic fever,[4] periarteritis nodosa [5] and other diseases.[6] The basic causal factor in all is related in some way to injury of the arteries. The effects on the renal tissues depend on the extent of distribution and the completeness of the occlusions. Accordingly, large or small portions of the kidneys are involved, the regions affected are single and large or multiple and small; and when

From the Henry Baird Favill Laboratory, St. Luke's Hospital.

1. Duff, G. L., and Murray, E. G. D.: Am. J. M. Sc. **201**:428, 1941. Weaver, R. G., and von Haam, E. M. M.: Arch. Int. Med. **63**:1084, 1939. Davidson, J.: Tr. Edinburgh Obst. Soc. **89**:101, 1929-1930.

2. (*a*) Klemperer, P.; Pollack, A. D., and Baehr, G.: Arch. Path. **32**:569, 1941. (*b*) Bunim, J. J.: Ann. Int. Med. **13**:1399, 1940. (*c*) Mook, W. H.; Weiss, R. S., and Bromberg, L. K.: Arch. Dermat. & Syph. **24**:786, 1931. (*d*) Libman, E., and Sacks, B.: Arch. Int. Med. **33**:701, 1924. (*e*) Contratto, A. W., and Levine, S. A.: New England J. Med. **221**:602, 1939. (*f*) Jarko, S.: Bull. Johns Hopkins Hosp. **59**:262, 1936. (*g*) Jager, B. V.: Arch. Dermat. & Syph. **46**:362, 1942. (*h*) Dine, C. H., and Wilson, C. S.: Am. J. Med. **1**:568, 1946.

3. (*a*) Talbott, J. H.; Gall, E. A.; Consolazio, W. V., and Coombs, F. S.: Arch. Int. Med. **63**:476, 1939. (*b*) Weiss, S.; Stead, E. A., Jr.; Warren, J. V., and Orville, T.: ibid. **71**:749, 1943. (*c*) Pollack, A. D.: Arch. Path. **29**:859, 1940.

4. (*a*) Hutton, R. L., and Brown, C. R.: Ann. Int. M. **20**:85, 1947. (*b*) Bruetsch, W. L.: J. A. M. A. **134**:450, 1947. (*c*) van der Horst, L.: Digest. Neurol. & Psychiat., Inst. of Living **15**:399, 1947.

5. (*a*) Singer, H. A.: Arch. Int. Med. **34**:865, 1927. (*b*) Manges, M., and Baehr, G.: Am. J. M. Sc. **162**:162, 1921. (*c*) Lamb, A. R.: Arch. Int. Med. **14**:481, 1914. (*d*) Keegan, J. J.: ibid. **36**:189, 1925. (*e*) Spiegel, R.: ibid. **58**:993, 1936. (*f*) Higgins, W. H.: South. M. J. **39**:453, 1946. (*g*) Pettit, H.: Am. Pract. **1**:333, 1947. (*h*) Massachusetts General Hospital Case, no. 32381, New England J. M. **235**:441, 1946. (*i*) Massachusetts General Hospital Case, no. 33181, ibid. **236**:670, 1947.

6. (*a*) Leiter, L.: J. A. M. A. **111**:570, 1938. (*b*) Fishberg, A. M.: Arch. Int. Med. **40**:80, 1927. (*c*) Derick, C. L., and Hass, G. M.: Am. J. Path. **11**:291, 1935. (*d*) Massachusetts General Hospital Case, no. 23041, New England J. Med. **216**: 170, 1937.

the occlusions initially are complete or extensive, renal function is reduced rapidly, death occurs, and the renal changes observed are those of acute necrosis. When the initial phase of the process of occlusion does not cause a sudden loss of functional renal tissues incompatible with living, other retrogressive changes occur in the involved tissues, such as atrophy, and in the portions with adequate blood supply, parenchymatous changes in the order of nephrosis. Kidneys with changes of this kind are herein described.

REPORT OF A CASE

A white woman aged 35 years had a migratory polyarthritis in the fifth month of her second pregnancy. The urine then contained only a trace of albumin, leukocytes and occasional erythrocytes. Shortly before she was delivered, her blood pressure was 162 systolic and 110 diastolic, and she had slight albuminuria. A full term live baby was born spontaneously. On the third day post partum the urine had a specific gravity of 1.016, a trace of albumin, no sugar, a few leukocytes and erythrocytes and many granular casts. Her blood pressure rose to a maximum of 184 systolic and 120 diastolic. Thirty-three days post partum she returned to the hospital because of cough, pain in the chest, dyspnea, hypertension, headaches and signs of cardiac decompensation. Two days later the sputum was stained with blood, and the right leg gave evidence of occlusion of the femoral artery. The urine now contained much albumin, many leukocytes and erythrocytes, and granular, waxy and cellular casts. The blood had 3,700,000 erythrocytes and 14,500 leukocytes per cubic millimeter. The following day she was transferred to the service of Dr. John T. Reynolds at St. Luke's Hospital. Circulation to the left leg was now also impaired. Decompensation of the heart was marked, and bilateral hydrothorax was present. The leukocytes were 26,150 per cubic millimeter; the clotting activity of the blood was 100 per cent, and the nonprotein nitrogen of the blood was 91 mg. per hundred cubic centimeters. On the second day, embolectomy of the right femoral artery was done, and spinal anesthesia was continued after the operation in the hope of improving circulation in the lower extremities. The patient continued in progressive cardiac and renal failure with decreasing urinary output and increasing evidence of uremia. She died in a convulsion on the third postoperative day and forty days post partum.

The essentials of the anatomic diagnosis of the postmortem examination (head, neck and trunk) were: subacute peurperal endometritis of the uterus; thrombosis of the veins of the broad ligament; mural thrombi of the left and right ventricles and auricles of the heart; bilateral extensive focal atrophy of the cortex of the kidneys; obturator thrombi of the left renal vein and of one of the two right renal veins and of the left and right internal iliac, left femoral and right profunda femoris arteries; recent femoral embolectomy wound of the right thigh; bilateral hydrothorax.

The uterus was slightly enlarged. Large portions of the endometrium of the upper part of the body were absent, and here the lining surface was granular. The endometrium remaining was gray and yellow, frayed, and 4 mm. thick. Veins of both broad ligaments were thrombosed. The heart weighed 380 Gm. In the right auricular appendage and in a pouch on the left side of the interatrial septum were small gray-red thrombi. Behind the posterior leaflet of the tricuspid valve were two gray mural thrombi 1 cm. in diameter and elevated 3 mm. Between the columnae carneae of the right ventricle were others. The left ventricle was dilated, and along the septum in front was a partially liquefied red-brown mural thrombus

3 cm. in diameter and 8 mm. thick. The leaflets of the heart valves had no changes. Cultures of the heart blood, the spinal fluid and the pericardial fluid were sterile.

The kidneys weighed, respectively, 155 and 165 Gm. The capsule of each stripped easily from a finely granular red-brown surface with scattered plateaus of a gray-yellow tissue, elevated 2 to 3 mm. above the red-brown portions (fig. 1). These elevated regions varied from 1 mm. to 2 cm. in maximum diameter and comprised about 45 per cent of the external surface. The gray-yellow tissues reached wedge-like through the cortex into the columns of Bertini, but not into the medulla, and

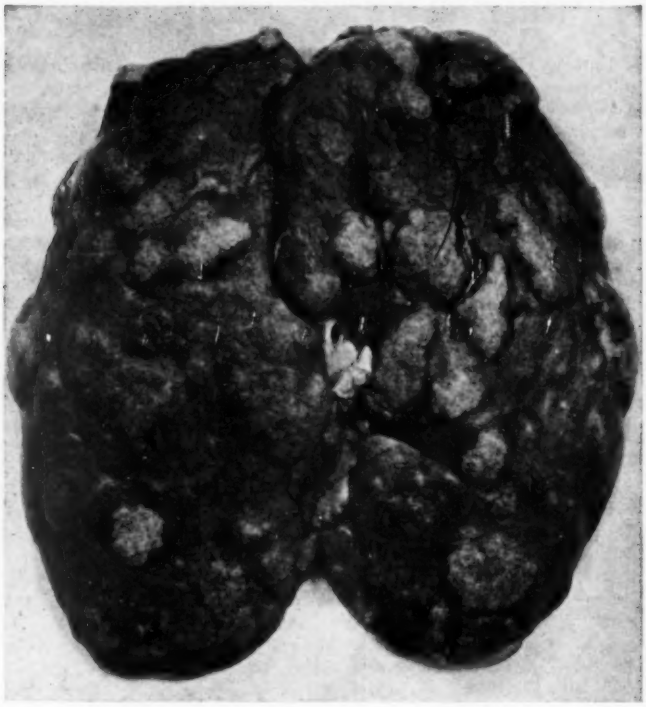


Fig. 1.—Subcapsular surface of the right kidney showing the depressed red-brown regions of cortical atrophy and the distribution of the plateau-like portions of gray nephrotic tissue.

had indistinct markings (fig. 2). The red-brown depressed tissues of the cortex were 5 mm. thick at the base of a pyramid. The left renal vein had an obturator thrombus 2 cm. long; the right kidney had two veins and the one to the upper pole contained an obturator thrombus which extended into the smaller branches. The renal pelves were not unusual.

Each pleural space contained about 800 cc. of a clear yellow fluid. The lungs were moderately hyperemic and edematous. Lymph nodes in the hilus of the left

lung had small encapsulated foci of caseous tissue. The liver weighed 2,250 Gm. Under the capsule were about a dozen gray tubercles, all less than 2 mm. in diameter. Surfaces made by cutting the liver were pale, red-brown, with distinct lobular markings and moderate fatty changes. The spleen weighed 140 Gm. and had about six small gray tubercles 2 mm. in diameter beneath the capsule and in the parenchyma. The lining of the stomach had a few petechial hemorrhages.

Histologic preparations of the endometrium of the uterus showed definite necrosis, which extended slightly into the myometrium. In many large arteries

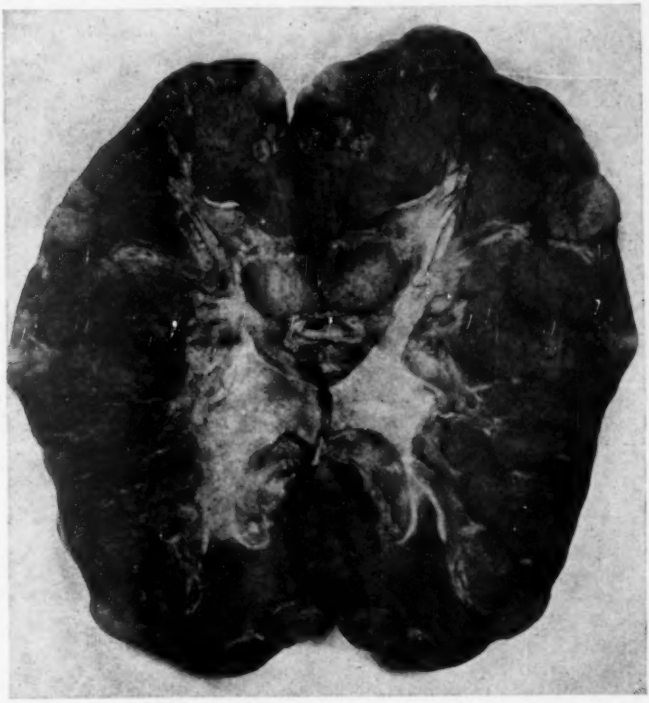


Fig. 2.—Surfaces made by hemisecting the left kidney, showing the wedge-shaped regions of gray nephrotic tissues extending through the cortex.

of the myometrium the intima was so thickened by fibrous tissues that the lumen was almost occluded. Others contained partially organized thrombi, and about them were exudates of lymphocytes and a few polymorphonuclear leukocytes. Many veins and some small arteries of the broad ligament also had thrombi in various stages of organization.

The mural thrombi of the left ventricle of the heart were partially organized. No masses of bacteria were found. In the underlying myocardium were a few lymphocytes and occasional polymorphonuclear leukocytes. Deeper in the myocardium were a few clusters of mononuclear cells about the blood vessels.

The renal tissues had two distinct patterns of structure. In the elevated portions, the glomeruli were large, and none was hyalinized (fig. 3 *A*). The afferent arterioles were not thickened, and the lumens of the vessels were patent. The

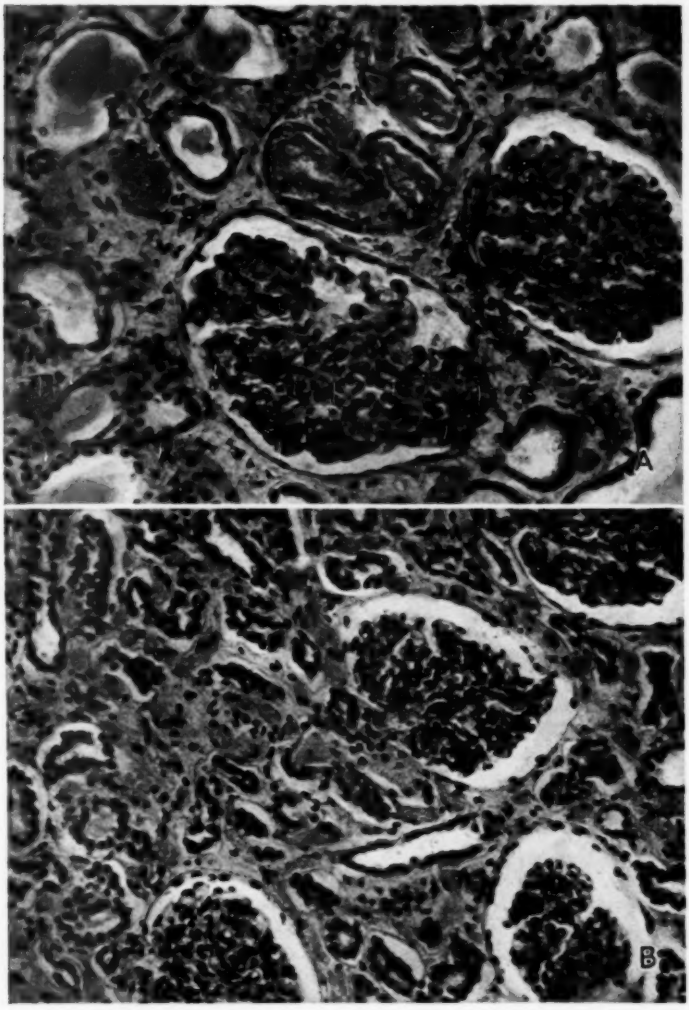


Fig. 3.—Photomicrograph illustrating the structure of (*A*) the elevated gray tissues of the kidney and (*B*) the depressed red-brown portions; × 198.

efferent arterioles were dilated with blood cells. Occasional intralobular arteries had a slight perivascular accumulation of lymphocytes, plasma cells and a few polymorphonuclear leukocytes. In sections cut serially some of the interlobular

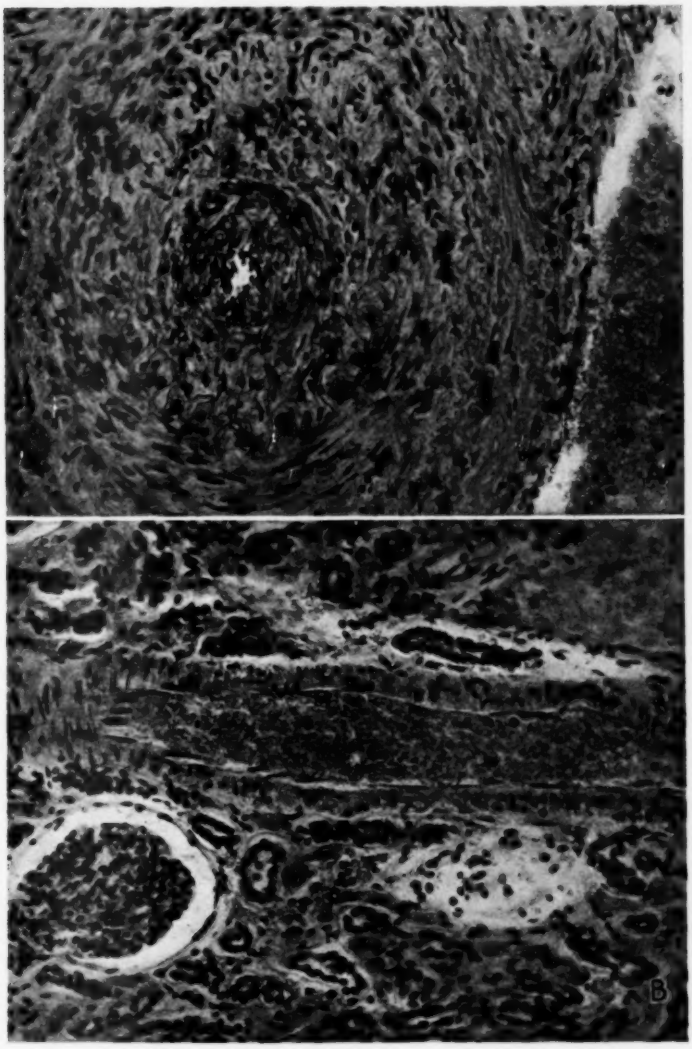


Fig. 4.—*A*, photomicrograph of an interlobular artery illustrating the extensive fibrous tissue thickening of the intima and the greatly reduced lumen.

B, photomicrograph of an intralobular artery in an atrophic portion of the kidney, illustrating the muscular wall and the widely patent lumen.

arteries had lumens partially occluded by fibrous tissue thickenings of the intima but had no perivascular inflammatory exudates and the internal elastic membrane was intact. A few of the convoluted tubules had flattened lining cells, but the others had columnar cells whose cytoplasm contained fine lipid deposits and distinct nuclei. Within the lumens of these tubules were granular precipitates. The collecting tubules had the usual low columnar lining cells, and their lumens contained amorphous precipitates. The depressed portions of the cortex contrasted sharply (fig. 3*B*). The glomeruli here were shrunken, the tufts were contracted and more cellular, and their capillaries were small. A few glomeruli were hyalinized. Bowman's capsule was unchanged. At all levels of the nephron the tubules were atrophic and in appearance resembled those of a fetal kidney. Accordingly, the number of atrophic glomeruli and tubules per unit area was greatly increased. The interstitial tissues were not appreciably increased. The afferent arterioles also had thin walls, and some of the intralobular arteries had perivascular collections of inflammatory cells but patent lumens. Many of the arcuate and interlobar arteries had fibrous thickenings of the intima ranging to complete occlusion (fig. 4*A*). Some were recanalized, and most of them had only a few mononuclear leukocytes in the wall. The intralobular branches had muscular walls but no occlusion (fig. 4*B*). Larger branches of the renal vein also had lumens occluded by organized thrombi.

There were a few small hemorrhagic infarcts in the lungs. The aorta showed slight fatty changes. A few of the vessels in the pancreas had small perivascular collections of lymphocytes. The blood vessels of other viscera were unchanged.

The changes of the kidneys were considered to be the result of disseminated fibrous tissue occlusions of the branches of the renal arteries which supplied the depressed red-brown regions of tissue and caused anemic atrophy of the parenchyma. The elevated gray-yellow tissues were regions spared or at least had a blood supply adequate to maintain structure.

Eight reports of similar focal atrophy of the kidney have been found in a review of the English literature. Mallory [6d] observed it in the kidneys of a man aged 48 years who died of coronary thrombosis. The medium-sized arteries of the kidneys had prominent hyaline fibrous tissue thickenings which greatly decreased the lumen. Mallory could not determine whether the changes were due to sclerosis or to endarteritis. This man had been treated with arsenical preparations for syphilis. Leiter [6a] described similar changes in the kidneys of a man aged 40 years in whom, after arsenical treatment for syphilis, hypertension rapidly developed. Although typical syphilitic cerebral endarteritis was present and spirochetes were demonstrated in necrotic duodenal tissues, Leiter was reluctant to consider the renal lesions as syphilitic, because they differed from the syphilitic nephritis reported by Volhard,[7] Wohlwill [8] and Rich.[9] He considered the possibility that the changes were an end result of a thrombotic process though not of visceral tromboangiitis obliterans. The renal lesions described by Talbott and associates, in a patient with

7. Volhard, F.: Handbuch der inneren Medizin, Berlin, Julius Springer, 1931, vol. 2, pt. 2, p. 1541.

8. Wohlwill, F.: Zentrabl. f. inn. Med. **47**:1066, 1926.

9. Rich, A. R.: Bull. Johns Hopkins Hosp. **50**:357, 1932.

dermatomyositis accompanied by scleroderma and calcinosis, were multiple regions of atrophy of the cortex. The intralobular arteries supplying these portions had great narrowing of the lumens caused by fibrous tissue thickenings of the intima. Some vessels had necrosis of the intima and inner portion of the media. Klemperer, Pollack and Baehr [2a] observed similar renal lesions in 3 of 35 cases of lupus erythematosus, but in appearance the lesions of the kidneys, they stated, resembled the focal scars of malignant nephrosclerosis. It is not clear from their description whether the changes in the kidneys were as extensive as those in the kidneys described in our report. However, they regarded them as due to vascular occlusions. Bunim [2b] also observed focal renal atrophy in lupus erythematosus, but in the patient, a woman aged 20 years, the advanced parenchymal atrophy was present only in the right kidney. Here large numbers of the interlobar and arcuate arteries had lumens partially or completely obliterated by dense connective tissue thickening of the intima and the elastic membrane and media were unaltered. No vascular lesions were seen in the left kidney. Hutton and Brown [4a] described rheumatic endarteritis of the renal vessels of a woman aged 31 years. The kidneys had not only regions of atrophy but also hyaline infarcts. The dense proliferations of collagenous fibrous tissue of the intima occurred not only in portions of the arcuate and larger arteries but also in the interlobular arteries. The myocardium contained Aschoff bodies and revealed endarteritis.

Other reports of similar lesions of the kidneys may have been published in foreign literature or in articles whose titles indicate neither this pathologic disorder nor the syndromes with which it has been found. A similar atrophy from intimal proliferation but usually associated with medial necrosis, aneurysmal dilatations and rupture of arteries producing also recent infarcts and hemorrhages has been described in periarteritis nodosa. Singer [5a] described the lesions in the kidneys of a man aged 57 years who died of chronic nephritis and hypertension. The capsular surface of the right kidney had gray elevations separated by gray-red depressed tissues. The blood vessels had thickened walls. Regions of atrophy of the cortical tissues alternated with regions in which the parenchyma appeared uninvolved. Although small aneurysms with medial necrosis were seen elsewhere, the arteries of the kidneys were not dilated. Other borderline examples have been described, and those in which an endarteritis produced hyaline scars. The latter occurred in a patient with lupus erythematosus observed by Mook and associates.[2c]

Atherosclerotic stenosis of a renal artery which greatly reduces the volume of blood flow causes atrophy of an entire kidney. This was observed in the body of a woman aged 62 years whose right renal artery had an ostium so narrowed by atherosclerosis that a 2 mm. probe was passed with difficulty. The shrunken right kidney weighed only 55 Gm.;

the left, with an unimpeded blood supply, weighed 145 Gm. The tissues of the right kidney were atrophic and like those in the red-brown depressed regions of the kidney described.

Probably the small number of published reports of these changes occurring in both kidneys indicates that they occur rarely. Those recorded have been associated with various diseases. In our case the lesions of both kidneys seem to be part of a complication of pregnancy in which puerperal endometritis, systemic venous and arterial thrombosis and thrombosis of the endocardium occurred. Cultural studies of the body fluids at the time of the necropsy, as well as a search of sections of the endocardial thrombi, failed to disclose bacteria. These negative results, however, do not exclude the presence of an infectious agent.

SUMMARY

Bilateral focal ischemic atrophy of the cortex of the kidneys caused by fibrous tissue or thrombotic occlusions of the intrinsic branches of the renal arteries has been reported in patients with lupus erythematosus disseminatus, scleroderma, rheumatic fever, periarteritis nodosa and possibly syphilitic endarteritis of the kidney. The disorder, according to our study, may occur as a complication of pregnancy.

Grossly, the atrophic portions are depressed red-brown tissues of the cortex, which contrast sharply with plateau-like regions of gray nephrotic renal tissues. The ischemic atrophy of the cortical tissues of the kidney results from a diminished flow of blood in the affected portions. The parenchymal changes in the atrophic portions are chiefly a decrease in the size of the various segments of the nephrons, which revert finally to structures resembling those in a fetal kidney.

The thrombotic and fibrous tissue occlusions of the intrinsic branches of the renal arteries are due to injury of these vessels. The nature of the noxious agent is not known, and may not be the same in the various diseases in which these lesions of the kidneys have been observed.

Renal functions, reduced progressively by the vascular lesions, become inadequate; symptoms of renal failure appear and death in uremia follows.

INFLUENCE OF AGE ON MAMMARY GROWTH AND INVOLUTION IN MALE MICE TREATED WITH ESTROGEN

RUTH SILBERBERG, M.D.
AND
MARTIN SILBERBERG, M.D.
ST. LOUIS

THE SUSCEPTIBILITY of male mice to estrogen-induced mammary cancer is partly determined by the age of the animal at the beginning of the treatment.[1] The lower incidence of such cancer in older animals has been attributed to a decreased responsiveness of the breast tissue and to an inhibiting effect exerted on mammary growth by the male sex hormone (androgen).[1] The present report deals with the microscopic changes observed in the noncancerous mammary glands of castrate and noncastrate mice receiving an estrogen at various ages. It was thought that such an investigation might throw some additional light on the role played by the age factor in estrogen-induced mammary growth.

MATERIAL AND METHODS

Sixty-five male mice of the closely inbred strain C3H, raised in our laboratory, were castrated at the age of 3 to 4 weeks. Twenty-three of these animals were given an injection of 0.03 mg. of alpha estradiol benzoate,[2] dissolved in sesame oil, once a week for five months from the age of 1 month on (younger age group); the remaining 45 animals received the same treatment from the age of 4 months on (older age group). Fifty-eight mice with intact testicles were given a similar course of injections, 26 animals from the age of 1 month on (younger age group) and 32 from the age of 4 months on (older age group). Details concerning the arrangement of the experiments, the technic employed, and the incidence of mammary cancers and lymphoid tumors in these mice have been published elsewhere.[1b,c] The present microscopic examination revealed two additional small carcinomas, one in a castrate and one in a noncastrate of the younger age group.

From the Snodgras Laboratory of Pathology, City Hospital.

The investigation was supported by a research grant from the National Cancer Institute, United States Public Health Service.

1. Loeb, L.: (*a*) Biol. Sympos. **11**:197, 1945. (*b*) Silberberg, M., and Silberberg, R.: Proc. Soc. Exper. Biol. & Med. **69**:438, 1948; (*c*) Arch. Path. **47**:340, 1949.

2. The Schering Corporation, Bloomfield, N. J., supplied the estrogen used, progynon-B.®

The resulting change in the tumor incidence, however, does not warrant a modification of the conclusions arrived at previously on the basis of the gross observations.[1b]

All animals except those in which palpable cancers or lymphoid tumors developed had been allowed to live to the end of their natural life. Not all of the available material could, however, be utilized. Some animals found dead had to be excluded from the microscopic study because of autolytic changes. Moreover, in order to satisfy the purpose of the present investigation, we could compare the breast tissues only of those animals that had lived for similar periods of time after discontinuation of the treatment.

Altogether, in the younger age group 26 mice (11 with intact testicles and 15 castrates) were used; in 4 of the former and 7 of the latter mammary cancer had developed. In the older age group 27 mice (9 with intact testicles and 18 castrates) were examined; mammary cancer had developed in 1 of the former and 7 of the latter (see tables 1 and 2). Usually 4 mammary glands were removed at necropsy, fixed in 4 per cent solution of formaldehyde on blotting paper to prevent curling, sectioned at several levels at 5 microns and the sections stained with hematoxylin and eosin.

MICROSCOPIC OBSERVATIONS

NONCASTRATE MICE TREATED WITH ESTROGEN FROM THE AGE OF ONE MONTH ON

Animals in Which No Breast Cancer Had Developed.—At the age of 8 to 11 months, that is, two to five months after discontinuation of the estrogenic treatment (fig. 1 *A*), ductal proliferation and secretion were accentuated, and in the acini, hyperplasia and hypertrophy, as well as secretion, were marked. Varying amounts of connective tissue were seen between the acini. At the age of 12 to 14 months, that is, six to eight months after the last injection (fig. 1 *B*), growth processes and secretion were definitely decreased as compared with the earlier stages in both ducts and acini; many acini were broken up, and there was abundant connective tissue surrounding the remaining epithelial elements. At the age of 15 months or over, that is, nine or more months after cessation of the treatment, the ducts and acini showed no hyperplastic or hypertrophic changes, and the resting state had recurred in 2 of 3 mice. Only an occasional globule of secreted material in a collapsed duct or in an acinus and a marked increase of fibrous tissue indicated that a previous stimulation of growth had taken place. In 1 animal, 17 months of age, active stimulation of the acinous growth was found, with the development of only small amounts of connective tissue.

Animals in Which Breast Cancer Had Developed.—The grossly noncancerous mammary glands showed advance stimulation of growth and pronounced secretion in the proliferating and dilated ducts and acini. There was neither involution nor decrease in the process of growth with an increasing interval after the last injection. A 14 month old mouse showed a small papillary cystadenoma in one mammary gland and an intraductal papilloma in another gland.

NONCASTRATE MICE TREATED WITH ESTROGEN FROM THE AGE OF FOUR MONTHS ON

Animals in Which No Breast Cancer Had Developed.—In ducts and acini of 8 to 11 month old mice there was but slight evidence of hyperplasia and hypertrophy of the epithelium. Some secretory globules were present, and the periacinous

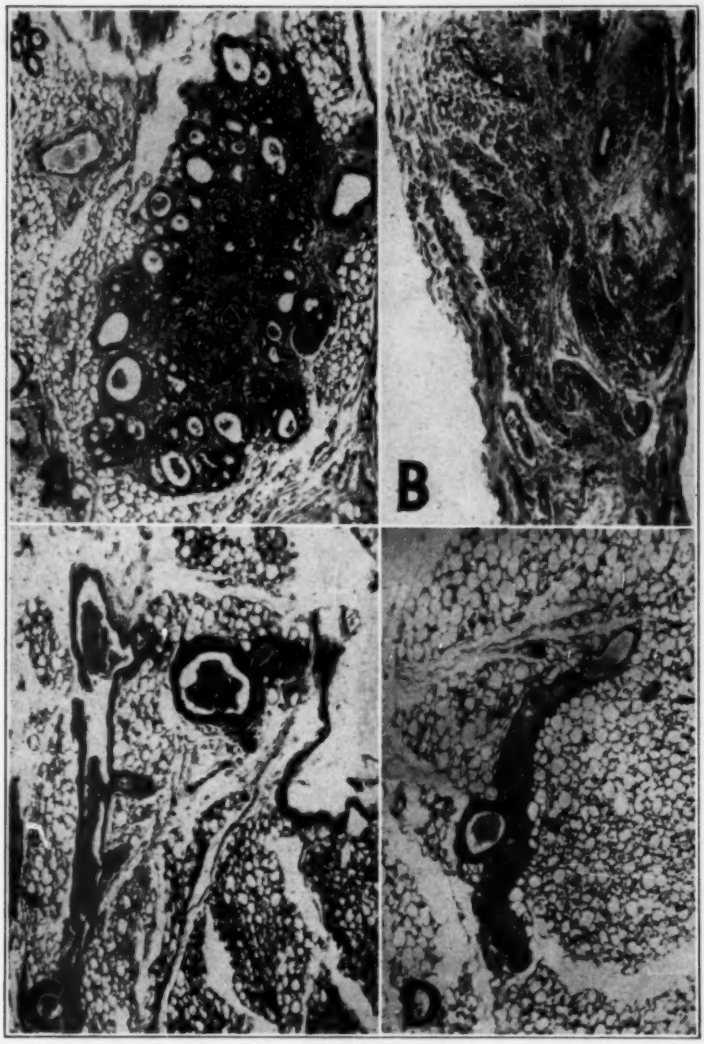


Fig. 1.—Sections through mammary glands of male mice of strain C3H: *A*, noncastrate male, 9 months old, treated with estrogen from the age of 1 month; tissue taken three months after the last injection; × 70. Ductal and acinous hyperplasia and secretion are seen. There is a moderate amount of connective tissue inside the lobule.

B, noncastrate male, 14 months old, treated with estrogen from the age of 4 months; tissue taken eight months after the last injection; × 70. A few ducts and isolated remnants of acini are seen. The lobular arrangement of the collapsed connective tissue can still be recognized, besides interlobular bands of dense connective tissue.

C, noncastrate male, 12 months old, treated with estrogen from the age of 4 months; tissue taken three months after the last injection; × 70. A few dilated ducts show some budding and contain secretion.

D, noncastrate male, 17 months old, treated from the age of 4 months; tissue taken eight months after the last injection; × 70. Note resting duct containing some secretion.

connective tissue was increased, particularly in 1 of the 3 animals. In mice 12 to 14 months old, that is, three to five mnoths after the last injection, the few acini present were in a resting state. The dilated ducts were lined by somewhat hypertrophic epithelium and contained some secretions (fig. 1 *C*). There was no increase of connective tissue. In the animals 15 months of age or older, in which the injections had been discontinued for six or more months before death, there was but slight dilatation of an occasional duct (fig. 1 *D*), which was surrounded by abundant adipose tissue. Acini were not found except in one mammary gland of 1 animal 20 months of age. In the latter a small adenomatous nodule was noted. In another mammary gland of the same mouse an intraductal papilloma was observed.

Animals in Which Breast Cancer Had Developed.—In the single animal in which a mammary cancer had developed at the age of 17 months, the ducts and acini of the grossly noncancerous mammary glands were distinctly hyperplastic and hypertrophic. One breast contained a cystadenoma of microscopic size. There was no evidence of involutionary changes.

CASTRATE MICE TREATED WITH ESTROGEN FROM THE AGE OF ONE MONTH ON

Animals in Which No Breast Cancer Had Developed.—In 8 to 11 month old mice, in which the estrogenic treatment had been discontinued since the age of 2 to 5 months (fig. 2 *A*), proliferation of both ducts and acini and secretion were advanced. In 1 animal 11 months of age there was slight fibrosis around the acini. At the age of 12 to 14 months (fig. 2 *B*), that is, six to eight months after cessation of the injections, ductal and acinous proliferation were of about the same order as at the earlier ages. The acini were, however, more dilated, and the epithelium was slightly lower than before. In mice 15 months of age or older the growth of the acinous epithelium was decreased, and the ducts had begun to collapse. The amount of secretion was likewise diminished, whereas the periacinous connective tissue was slightly increased.

Animals in Which Breast Cancer Had Developed.—The grossly noncancerous mammary glands showed at all ages active secretion and advanced stimulation of growth of both ducts and acini. In 3 animals, 9, 14 and 16 months old, microscopic benign intraductal papillary tumors were observed. An additional animal, 17 months old, had a carcinoma of microscopic size in one mammary gland. Involutionary changes were missing or slight at best.

CASTRATE MICE TREATED WITH ESTROGEN FROM THE AGE OF FOUR MONTHS ON

Animals in Which No Breast Cancer Had Developed.—In the single mouse examined at 10 months of age, that is, one month after the last injection, epithelial proliferation was quite marked in ducts and acini, and there was abundant secretion. No involutionary changes were noted. In animals 12 to 14 months of age, in which the injections had been discontinued for three to five months, respectively (fig. 2 *C*), there were clusters of small acini scattered in the adipose tissue. The lining epithelium was low cuboidal, and secretion was present. The ducts showed some stimulation of growth. In most of the animals 15 months of age or older, in which injections had been discontinued for six or more months

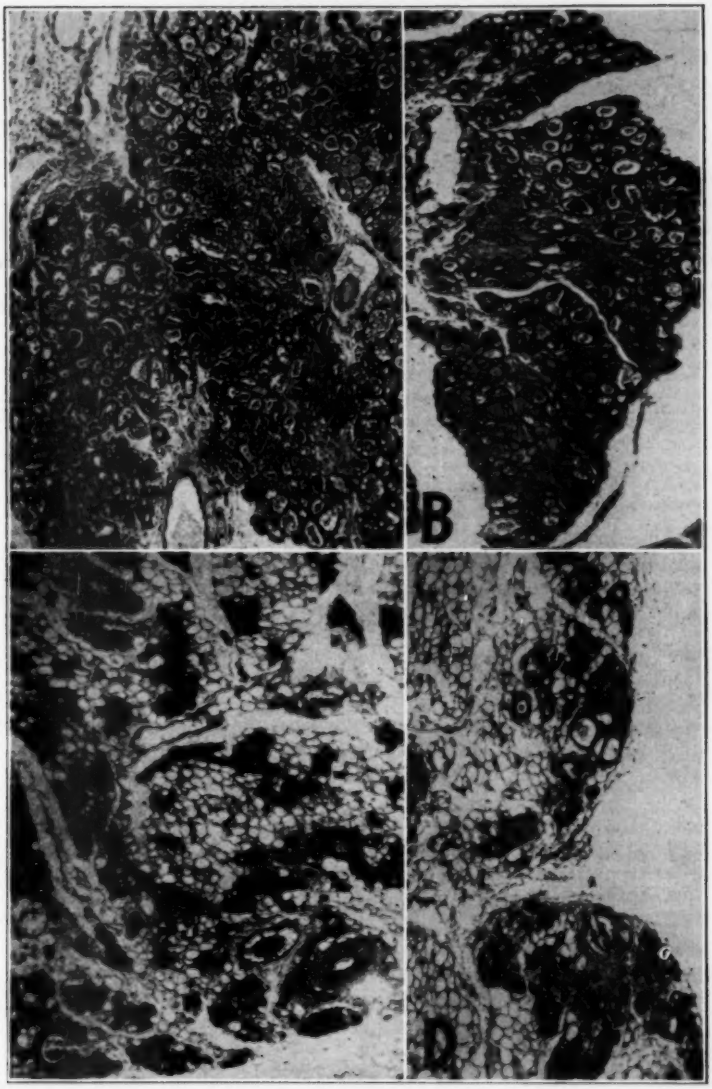


Fig. 2.—Sections through mammary glands of male mice of strain C3H: *A*, castrate male, 9 months old, treated with estrogen from the age of 1 month; tissue taken three months after the last injection; × 70. This is a large mammary nodule composed of densely packed acini. The lumens of the acini are lined by hyperplastic epithelium and contain varying amounts of secretions. Little connective tissue is present between the acini.

B, castrate male, 14 months old, treated with estrogen from the age of 1 month; tissue taken eight months after the last injection; × 70. Mammary nodules are seen, composed of densely packed acini containing secretions and lined by hyperplastic epithelium. There is no evidence of involutionary changes.

C, castrate male, 12 months old, treated with estrogen from the age of 4 months; tissue taken three months after the last injection; × 70. Scattered groups of small acini are seen in the fat tissue.

D, castrate male, 17 months old, treated with estrogen from the age of 4 months; tissue taken eight months after the last injection; × 70. Few clusters of small acini and some ducts are seen. There is no evidence of involutionary changes.

before death (fig. 2*D*), ductal and acinous growth was accentuated. A few dilated acini contained secreted material. The epithelial lining of ducts and acini was cuboidal and inactive. In 2 of the 8 animals the acinous proliferation was more diffuse and more active than in the rest of the mice. In 1 of these 2 animals

TABLE 1.—*The Degrees of Proliferation, Secretion and Involutionary Changes Observed in the Mammary Glands of Noncastrate Male Mice*

	Younger Age Group (Treated with Estrogen from the Age of 1 Month on)						Older Age Group (Treated with Estrogen from the Age of 4 Months on)					
Age at death, mo.	8-11		12-14		15+		8-11		12-14		15+	
Interval since last injection, mo.	2-5		6-8		9+		0-2		3-5		6+	
	No Cancer	Cancer	No Cancer	Cancer*	No Cancer	Cancer	No Cancer	Cancer	No Cancer	Cancer	No Cancer†	Cancer
Cases	2	2	2	1†	3	1	3	0	2	0	3‡	1*
Proliferation: Ducts	1, 2	2, 3	1, 2	3	1	3	1	..	1	..	0, 1§	2
Proliferation: Acini	2	2, 3	0, 1	3	0, 2‖	3	1	..	0	..	0, 1§	2
Secretion in ducts and acini	2	3	2, 1	3	0, 1	3	2	..	1	..	0, 2	2
Involutionary change	1	0	3	0	3, 1¶	0	1, 2‖	..	0	..	0	0

* Papillary cystadenoma occurred in a noncancerous mammary gland.
† One animal showed an intraductal papilloma in a noncancerous mammary gland.
‡ An adenomatous nodule was seen in the mammary gland of a 20 month old animal.
§ Grade 1 was observed in 1 animal.
‖ Grade 2 was observed in 1 animal.
¶ Grade 3 was observed in 2 animals.

TABLE 2.—*The Degrees of Proliferation, Secretion and Involutionary Changes Observed in the Mammary Glands of Castrate Male Mice*

	Younger Age Group (Treated with Estrogen from the Age of 1 Month on)						Older Age Group (Treated with Estrogen from the Age of 4 Months on)					
Age at death, mo.	8-11		12-14		15+		8-11		12-14		15+	
Interval since last injection, mo.	2-5		6-8		9+		0-2		3-5		6+	
	No Cancer	Cancer	No Cancer	Cancer	No Cancer	Cancer*	No Cancer	Cancer	No Cancer	Cancer	No Cancer	Cancer
Cases	2	2*	4	3*	2	2†	1	3	2	2	3*	2‡
Proliferation: Ducts	2	2, 3	2	2, 3§	2	2, 3	2	3	1	2	1	2
Proliferation: Acini	2, 3	3	2, 3§	2, 3‖	1, 2	3	2	2, 3§	1, 2	3	1, 2¶	2
Secretion in ducts and acini	3	3	2	3	1, 2	2, 3	2	3	1	3	1	2, 3
Involutionary change	0, 1	0, 1	0, 1#	0	1, 2	0, 1	0	0	0	0	0, 1#	2¶, 0

* Intraductal papilloma was observed in 1 animal.
† A microscopic carcinoma was observed in one grossly noninvolved mammary gland.
‡ A microscopic carcinoma was observed in two grossly noninvolved mammary glands of the same animal.
§ Grade 3 was observed in 1 animal.
‖ Grade 3 was observed in 2 animals.
¶ Grade 2 was observed in 2 animals.
# Grade 1 was observed in 1 animal.

the ductal epithelium showed foci of papillary projections. On the whole, there was little or no fibrosis, although in 2 of the 8 mice somewhat larger amounts of periacinous connective tissue were observed.

Animals in Which Breast Cancer Had Developed.—In all 7 animals the noncancerous breast tissue was in an advanced state of proliferation and showed

hypertrophy with abundant secretion in ducts and acini. The stimulation of growth was most pronounced in the animals 8 to 11 months old; it was somewhat less accentuated in the older mice. There was no evidence of a return to a resting state. In 1 mouse, 9½ months of age, diffuse adenosis was noted in all glands. Two other animals, 11 and 14 months old, respectively, showed marked stimulation of acinous growth in one noncancerous gland but less in the other two breasts. In a 19 month old mouse, two grossly noninvolved mammary glands had undergone early carcinomatous change.

The changes found in the various groups were graded and are summarized in tables 1 and 2.

A schematic tabulation has the disadvantage that certain variations of detail cannot be properly recorded. However, such a presentation facilitates a comparison of the main findings, particularly if a larger number of experimental groups is involved. Mammary growth processes, amount of secretion and involutionary changes were graded and included in the tables. The three grades of stimulation of growth used are essentially the same as those used by Loeb and Suntzeff.[3] Grade 0 designates the resting state.

Changes in the Ducts: Grade 1 indicates focal increase in number and size of the lining cells with an occasional mitotic figure; grade 2 is characterized by diffuse proliferation and hypertrophy of the epithelium associated with marked dilatation, elongation and budding of ducts; changes marked as grade 3 consist of pronounced proliferation and hypertrophy of the epithelium, more frequent mitotic figures, hyperchromasia of the nuclei and infolding of the epithelium into the ductal lumen.

Changes in the Acini: Grade 1 is characterized by focal increase in the number of acini; grade 2 indicates more widespread new formation of acini with mitotic proliferation and hypertrophy of the epithelium, whereas in grade 3 there were numerous closely packed hyperplastic and hypertrophic acini supported by thin strands of connective tissue.

Secretion: Grade 1 designates the presence of isolated shreds of eosinophilic material in the ductal lumen and small intracellular vacuoles; grade 2, thick globules of eosinophilic material in ducts and large secretory vacuoles in the lining cells; grade 3, occlusion of many enlarged ducts and acini by abundant eosinophilic secretions.

Involutionary Changes: Grade 1 indicates decrease in size and number of the acini with solution of the secretions and slight increase of the periacinous connective tissue; grade 2 designates collapse and fragmentation of acini with releasing of secretions into the supporting stroma, phagocytosis of secreted material and further increase of connective tissue; grade 3 is characterized by the presence of connective tissue arranged in a lobular fashion and rarely containing an atrophic epithelial cell. The latter condition may be spoken of as collapse fibrosis. The supporting connective tissue, which had increased during the period of stimulation of growth, remained preserved for longer periods than the epithelium, and it still appeared increased in amount when the epithelium had undergone regression. This increase was, however, relative rather than absolute, the connective tissue becoming conspicuous because of the involution of the glandular elements. At later stages of involution this fibrous tissue was in turn infiltrated by adipose tissue.

3. Loeb, L., and Suntzeff, V.: Arch. Path. **32**:730, 1941.

COMPARISON OF THE CHANGES IN NONCASTRATES OF THE YOUNGER AND OLDER AGE GROUPS

Animals in Which No Breast Cancer Had Developed.—On the whole, the mice of the younger age group showed more marked stimulation of mammary growth than those of the older age group. Only in 1 of the 8 animals of the latter group was there found a small conglomeration of acini (stimulation of grade 2). In the younger group 3 of the 7 mice showed grade 2 proliferation of the acini. The intensification of growth in the younger age group seems even more significant in view of the fact that animals of a given age in the older group were actually three months closer to the last injection than animals of corresponding age of the younger group. In other words, any involutionary changes that might counteract the effects of preceding stimulation of growth would have acted three months longer in the younger than in the older age group. Actually, in the younger series the processes of involution increased with increasing interval after the last injection, whereas in the older age group involutionary changes were less pronounced at all stages after discontinuation of the treatment. Therefore, the conclusion seems justified that, from the beginning on, the stimulation of growth was by far less accentuated in the older group than in the younger series.

Animals in Which Breast Cancer Had Developed.—The growth stimulation of the noncancerous glands of the 1 animal of the older age group was less marked (grade 2) than that observed in the younger age group (grade 3).

COMPARISON OF THE CHANGES IN NONCASTRATES AND CASTRATES OF THE YOUNGER AGE GROUP

Animals in Which No Breast Cancer Had Developed.—At any given age the stimulation of ductal and particularly of acinous growth and secretion was more impressive in the castrates than in the animals with intact testicles. This became more obvious as the interval between the last injection and the death of the animal increased: In 5 of the 6 castrates over 12 months of age there was still active (grade 2 or 3) ductal and acinous growth and secretion; of the 5 noncastrate mice of corresponding age, only 1 showed grade 2 acinous stimulation, whereas in the remaining 4 animals a return to a resting or almost resting state had occurred. Thus, involution was more rapid and more complete in the noncastrates than in the castrates.

Animals in Which Breast Cancer Had Developed.—There was no appreciable difference in the reaction of the mammary glands of castrates and noncastrates, both showing intense ductal and acinous stimulation. This applied to the general processes as well as to the more localized formation of benign intraductal papillae.

COMPARISON OF THE CHANGES IN CASTRATES AND NONCASTRATES OF THE OLDER AGE GROUP

Animals in Which No Breast Cancer Had Developed.—In the castrates, as a rule, epithelial growth, although not exceeding grade 2, was still more active than in the noncastrate animals. Of 8 noncastrate mice, only in 1 animal, 20 months old, was stimulation of acinous growth of grade 2 noted in one of the four mammary glands. Involutionary changes were delayed in the castrates as compared with the noncastrates, and in the latter such changes were seen only at early stages after cessation of the estrogenic treatment. The absence of these involutionary processes in noncastrate mice over 12 months of age is in agreement with the fact

that in this group the maximum stimulation had not exceeded grade 1 except in 1 mouse. Involutionary changes cannot be expected to be conspicuous in glands in which there had been only slight stimulation of growth.

Animals in Which Breast Cancer Had Developed.—In the 1 noncastrate in which a mammary cancer had developed, conditions were essentially similar to those in the castrates bearing mammary gland carcinoma.

COMPARISON OF THE CHANGES IN CASTRATES OF THE YOUNGER AND OLDER AGE GROUPS

Animals in Which No Breast Cancer Had Developed.—In mice of the younger age group the degree of stimulation of the breast tissue was rather uniform up to eight months after the last injection. Subsequently there was a slight decline of growth and correspondingly there was some increase in the involutionary changes. In the older age group the maximum stimulation was one degree lower than that in the younger age group. This difference may be, however, more significant than it appears, since again animals of the older age group were three months nearer to the last injection at the time of their death than those of identical age of the younger age group. Processes of involution were slight in the younger and practically absent in the older age group; in the latter, only 2 of the 8 mice over 15 months of age showed an involutionary change of grade 2.

Animals in Which Breast Cancer Had Developed.—There was in the two age groups of castrates hardly any difference in the degree of stimulation of the grossly noncancerous mammary glands.

COMPARISON OF THE CHANGES IN THE BREASTS OF MICE BELONGING TO DIFFERENT AGE GROUPS BUT LIVING FOR A COMPARABLE PERIOD OF TIME AFTER THE LAST INJECTION

The interval which has elapsed since the last injection is of importance for the appraisal of the changes taking place in the mammary gland: During this time, either growth processes may increase, resulting in cancerous growth, or involutionary processes may set in and counteract the preceding preparatory stimulation of growth, thus preventing the transition from the preparatory to the cancerous phase and leading to a restoration of the resting state. In order to evaluate the effect of age on the course of growth after the cessation of the injections, it seemed necessary to supplement the foregoing tables by a comparison of animals of both age groups living for a comparable period after the last estrogenic injection. Animals of the older series 12 to 14 months of age should therefore be compared with mice of the younger series 8 to 11 months old, and animals of the older series 15 and more months of age should be compared with those of the younger series 12 to 14 months old.

Animals in Which No Breast Cancer Had Developed.—Animals with Intact Testicles: Stimulation of mammary growth was distinctly less accentuated in the older than in the younger age group: Three to five months after the last injection the mice of the older group 12 to 14 months of age showed no acinous and only slight ductal proliferation, whereas acinous growth of grade 2 was present in the 8 to 11 month old animals of the younger age group. Involutionary changes were lacking in the animals of the older group, whereas they were present, though slight, in the younger series. As discussed in a foregoing section, this indicates that in the older group the stimulation of growth had been less pronounced than in the younger group from the beginning and that therefore less tissue could

undergo involution. A comparison of the findings in mice of the older age group 15 or more months of age and those of the younger age group 12 to 14 months of age—both killed six or more months after the last injection—led to similar results: In the younger age group there was more breast tissue present, and involutionary processes were more marked, than in the older age group.

Castrate Animals: In mice of the older age group 12 to 14 months old and living three to five months after cessation of the estrogenic treatment, ductal and acinous growth showed a stimulation of grade 1 or 2; in the mice of the younger age group 8 to 11 months old and living for three to five months after the last injection, the growth processes were those of grade 2 or 3. Correspondingly, most mice of the older group (6 of 8 animals) 15 or more months old and living six or more months after the last injection showed stimulation of mammary growth of grade 1, whereas in the 4 castrates of the younger age group 12 to 14 months old and living six and more months after the last injection the stimulation of growth was of grade 2 or 3. Involutionary cnanges in all groups of castrates reached a noticeable degree only at later stages after discontinuation of the estrogenic treatment.

Animals in Which Breast Cancer Had Developed.—The degree of growth stimulation in the grossly noninvolved mammary glands was about the same in the two age groups.

COMMENT

The results of the comparisons made in the preceding paragraphs may be summarized as follows:

Alpha estradiol benzoate injected from the age of 1 month intensified mammary growth of male mice of strain C3H with intact testicles. If administration of the estrogen was begun at 4 months of age, mammary growth was by far less stimulated than in the younger age group. This effect is in agreement with the different incidence of mammary carcinoma in these mice whose treatment began at different ages (15.8 per cent in the younger and 4 per cent in the older age group [1b]). Moreover, in the noncastrates the mammary glands rapidly returned to a resting state after discontinuation of the injections. The difference in the responsiveness of the mammary glands of the two age groups might be due to the presence of the testicles, to an age factor or to both.

In male castrates of strain C3H the estrogen caused a more marked intensification of mammary growth than in the corresponding animals with intact testicles, irrespective of the age at which the injections were begun. Likewise, after discontinuation of the injections the involutionary processes proceeded more slowly in castrates than in noncastrates. The antagonistic effect of the testicular hormone (androgen) on estrogen-induced growth of the mammary gland [4] could thus be confirmed.

4. Lacassagne, A., and Raynaud, A.: Compt. rend. Soc. de Biol. **131**:186, 1939. Nathanson, I. T., and Andervont, H. B.: Proc. Soc. Exper. Biol. & Med. **40**:421, 1939. Jones, E. E.: Cancer Research **1**:787, 1941. Heiman, J.: ibid. **4**:31, 1944. Gardner, W. U.: ibid. **6**:493, 1946.

In castrate males of strain C3H treated from the age of 1 month on, the growth stimulation of the mammary gland reached a higher degree than in castrates receiving the estrogen from the age of 4 months on. An age factor independent of the testicle thus aids in determining the response of the mammary gland to estrogen not only as far as carcinogenesis is concerned but also as regards the progress of the preparatory period of growth. In castrates of both age groups, however, there were only slight and slowly progressing involutionary changes in the acini as the interval from the last injection to the death of the animal increased. The age factor seems, therefore, to play a minor role in the progress of involution of the mammary glands stimulated previously by the estrogen.

TABLE 3.—*The Relative Significance of the Age Factor and the Testicular Hormone as Antagonists of Estrogenic Stimulation of the Mammary Gland*

	Interval After Last Injection	Ratio of Acinous Growth in:	Indicates Role of:
		Young Noncastrate : Old Noncastrate	
I.	2-5 mo.	2 : 0	Testicular Hormone and Age Factor
	6-8 mo.	½ : 0	
		Castrate Young : Noncastrate Young	
IIa.	2-5 mo.	2½ : 2	
	6-8 mo.	2¼ : ½	
	9+ mo.	1⅓ : ⅔	Testicular Hormone
		Castrate Old : Noncastrate Old	
IIb.	0-2 mo.	2 : 1	
	2-5 mo.	1½ : 0	
	6-8 mo.	1¼ : 0	
		Young Castrate : Old Castrate	
III.	2-5 mo.	2½ : 1⅙	
	6-8 mo.	2¼ : 1¼	Age Factor

Table 3 illustrates the relative significance of the age factor and the testicular hormone as regards their inhibiting effects on estrogen-induced growth of the mammary gland. It represents a condensation of tables 1 and 2 with only the grades of acinous growth shown, for the sake of simplification. The figures were arrived at by adding the grades of acinous stimulation found in the individual animals and dividing the total by the number of animals in the respective groups. In view of the difficulty of expressing microscopic changes in numerical terms, the tabulation should be considered as an aid in discerning trends rather than as a basis for an exact statistical analysis.

The most powerful inhibition of mammary growth (horizontal column I) was exerted by the combined action of both the testicle and the age factor, the latter presumably being due to constitutional factors inherent in the breast tissue itself as pointed out by Loeb.[1] The part played by the testicular hormone in the inhibition of estrogen-induced mammary growth is indicated in horizontal columns IIa and IIb.

At the time of the maximum stimulation, two to five months after the last injection, there was but one-half degree difference between castrates and noncastrates of the younger group (column IIa) and one full degree between castrates and noncastrates of the older age group (column IIb). The inhibiting effect of the testicular hormone appears, therefore, to be comparatively slight. However, if the changes of acinous growth seen with increasing interval from the last injection (vertical column in horizontal column IIa and IIb) are considered, the marked influence of the male sex hormone (androgen) becomes more evident. The return to the resting state was accelerated in the presence of the testicle, suggesting that the latter is largely responsible for the progress of involutionary changes in the mammary glands of those mice in which no cancer had developed. The inhibitory effect exerted on mammary growth by the age factor (horizontal column III) approximates that exerted by the testicle during the early stages following treatment. However, the age factor failed to influence significantly the progress of involutionary processes.

In the comparatively few noncastrate animals in which mammary cancer developed, the mammary gland tissue must have been sufficiently responsive or the stimulus acting on the acini must have been powerful enough to overcome the restraining influence of both the testicular androgen and the age changes taking place in the mammary gland tissue. This diminution of resistance was confirmed by the microscopic appearance of the noncancerous mammary glands. In the latter the growth processes were distinctly stimulated, while involutionary changes were absent or insignificant.

SUMMARY

In castrate and noncastrate male mice of strain C3H treated with an estrogen the growth of the mammary gland was stimulated even in those animals in which mammary cancer did not develop. The intensification of growth processes paralleled closely the incidence of breast cancer in castrate and noncastrate mice treated with the estrogen. In castrates of a given age the mammary growth was more enhanced than in noncastrates of a corresponding age, and in castrates as well as in noncastrates receiving the estrogen during earlier periods of life the growth stimulation exceeded that seen in animals treated similarly at later ages. Processes of involution proceeded more rapidly in the presence of the testicles irrespective of the age of the animal at the time of the treatment.

Thus at least two factors are involved in the inhibition of estrogen-induced growth of the male breast: (1) the male sex hormone (androgen) which inhibits mammary growth during estrogenic treat-

ment and promotes involutionary processes after discontinuation of the treatment; (2) an age factor inherent in the aging breast tissue and opposing the stimulation of growth caused by the estrogen; in the involution of the stimulated mammary gland the role of this age factor is less conspicuous than that of the testicle.

The noncancerous glands of the mice in which breast cancer had developed showed a high degree of stimulation throughout. These findings, in conjunction with those obtained in the mammary glands of noncancerous mice, suggest that factors which promote or inhibit cancerous growth also intensify or oppose those phases of growth that precede cancer formation.

GASTRIC PARAGANGLIOMA WITH ULCERATION

Report of a Case

CHESTER K. JONES, M.D.
AND
FRANK W. McKEE, M.D.
ROCHESTER, N. Y.

THE PURPOSE of this paper is to report a paraganglioma occurring in the wall of the stomach, to review the literature and to remark on the existing lack of agreement in classification and terminology concerning this type of tumor.

Chromaffinoma, or paraganglioma, was first described, according to Wahl,[1] in 1883 by Weichselbaum, and similar tumors were recorded by Frankel in 1886 and by Perley in 1890, according to Lewis and Geschickter.[2] Reid[3] cited Berdez as having reported a case in 1892. Kohn is credited with having published the first description of the chromaffin system in 1902. He included the carotid body, the gland at the aortic bifurcation, the medulla of the adrenal gland and the vertebral sympathetic ganglions. Subsequent authors[4] made additions or deletions in this original definition of the chromaffin system. Recent papers[5] have tended to separate the carotid body tumor and the carcinoid, or argentaffinoma, from the paraganglioma.

The embryology of the paraganglion cells, which give rise to these tumors, deserves a word. The neural crest gives rise to immature cells which migrate along the sympathetic nervous system. Some come to rest in the form of small masses in concavities on the dorsal surfaces of the sympathetic ganglions; hence the term "paraganglions." Before these cells mature into the adult chromaffin cells, other primordial sympathetic nerve cells migrate to the adrenal medulla, the abdominal sympathetic plexuses, the organs of Zuckerkandl and elsewhere in the autonomic nervous system. These sympathetic cells have been described in the thorax, the retroperitoneal tissues, the renal hilus, sacrococcygeal

From the Department of Pathology, Genesee Hospital.

1. Wahl, H. R.: J. M. Research **30**:205, 1914.
2. Lewis, D., and Geschickter, C. F.: Arch. Surg. **28**:16, 1934.
3. Reid, M. R.: Ann. Surg. **88**:516, 1928.
4. Guild, S. R.: Anat. Rec. **79**:28, 1941. Lewis and Geschickter.[2]
5. (*a*) Brines, A. O., and Jennings, E. R.: Am. J. Path. **24**:1167, 1948. (*b*) Waaler, E.: Acta med. Scandinav. **123**:1, 1945. (*c*) Philips, B.: Arch. Path. **30**:916, 1940.

tissue, the celiac ganglion, the breast, the arm, the tibia and the upper part of the sternum.[6] Bielschowsky[7] stated in his discussion of the embryogenesis of the sympathetic ganglions that the primitive neurocytes or sympathogonia are the mother cells not only of the sympathoblasts and sympathetic ganglion cells but also of the chromaffin cells. The dichotomous division of the "family tree" as presented by Bielschowsky is shown in table 1. The corresponding tumor is inserted with its cell of origin.

Bielschowsky added that the genealogy of the sympathogonia is not sharply defined inasmuch as certain tumors reveal elements of both neuroblastic and pheochromic characteristics. Wahl[1] in 1914 recognized this fact, stating that examples of one pure type are rare. He reported a case illustrating three anatomically distinct types. Later, in 1943, in conjunction with Robinson, Wahl[8] reported a case of neuroblastoma of the mediastinum with a pheochromoblastomatous element. The latter tumor is described as showing neurocytes, ganglion cells, nerve fibrils, pheochromoblasts and pheochromocytes. In 1939 Potter and Parrish[9] reported a 2 month premature infant which at autopsy revealed a tumor made up of sympathogonia, sympathoblasts and ganglion cells. An anatomically separate schwannoma was also found in this infant. To complete the consideration of the vagaries of which these tumors of sympathetic system origin are capable, the case reported by Cushing and Wolbach[10] in 1927 should be mentioned, in which there was transformation of a cancerous paravertebral sympathoblastoma into a benign ganglioneuroma.

TABLE 1.—*Dichotomous Division of the "Family Tree" of the Sympathogonia According to Bielschowsky*[7]

Sympathogonia (Sympathogonioma)		
Sympathoblast (Sympathoblastoma)		Pheochromoblast (Pheochromoblastoma)
Sympathetic Ganglion Cell (Ganglioneuroma)		Pheochromocyte or Chromaffin Cell (Pheochromocytoma or Paraganglioma)

Chromaffin tumors are rare. According to Brines and Jennings,[5a] approximately 210 have been reported. The terms "paraganglioma,"

6. Sailer, S.: Am. J. Path. **19**:101, 1943.

7. Bielschowsky, M., in Penfield, W.: Cytology and Cellular Pathology of the Nervous System, New York, Paul B. Hoeber, Inc., 1932, p. 1083.

8. Wahl, H. R., and Robinson, D.: Arch. Path. **35**:571, 1943.

9. Potter, E. L., and Parrish, J. M.: Am. J. Path. **15**:652, 1939.

10. Cushing, H., and Wolbach, S. B.: Am. J. Path. **3**:203, 1927.

"chromaffin tumor" and "pheochromocytoma" are found to be used interchangeably in the literature, various authors employing the same term to denote histologically different tumors. Henceforth, we shall speak of paraganglioma as Pick[11] first defined the tumor in 1912, those chromaffin tumors occuring without the adrenal gland, in contrast to those which occur within the adrenal gland, which are designated pheochromocytoma. Waaler[5b] collected all the cases of paraganglioma in the literature up to 1945. He cited Gormsen's review with 20 cases of paraganglioma and added 18 of his own. On reviewing the literature since 1945 one notes that approximately 8 additional cases, including our own, of extra-adrenal paraganglioma have been reported.

REPORT OF CASE

A 38 year old white man, employed as a commercial chauffeur, was admitted to the Genesee Hospital, Rochester, N. Y., Nov. 8, 1948, with the chief complaint of heaviness and pain in the stomach of two years' duration. The patient stated that two years previously he began to note postprandial heaviness and discomfort in his stomach, which often lasted for several hours. Several months later, the gastric distress, described as a dull pain in the pit of the stomach, became preprandial, and was relieved by food. Approximately one year before admission, the pain became postprandial again, appearing one-half to one hour after meals. This dull postprandial pain, together with a continually increasing feeling of gastric heaviness, persisted. The patient recalled one large rectal hemorrhage and that subsequently he had observed dark stools for a period of two months prior to admission. During the course of his illness, the patient had been treated by four or five different physicians for peptic ulcer and had found that milk relieved his pain temporarily. He suffered a 20 pound (9 Kg.) loss of weight during the year prior to admission, together with occasional weakness and lethargy.

At the time of admission the temperature was 99 F., the pulse rate 76, the respiratory rate 20 and the blood pressure 130 systolic and 80 diastolic. There was slight epigastric tenderness to deep palpation. No masses were felt. The patient's hemoglobin was 12.1 Gm. per hundred cubic centimeters; the red blood cell count was 4,200,000. The radiologist Dr. G. J. Baron reported on a gastrointestinal series made Nov. 8, 1948 as follows: "No abnormalities are noted in the esophagus. The stomach in general shows a good outline with perhaps slight hypertrophy of the rugae. Peristalsis is quite active in the proximal portions of the stomach. In the pyloric antrum there is a large, smooth, quite well defined filling defect with a broad base, which appears to arise from the lesser curvature. The outline of this defect is not particularly irregular, and rugal markings can be seen in the filled portion of the stomach in this area. There does not appear to be significant obstruction, barium sulfate passing freely into the duodenal bulb, which shows normal outline and activity. No abnormality is noted in the duodenal loop or the upper part of the small bowel." The interpretation of the finding was that it might be a benign polyp but that more likely it was a cancer, because of the broad base. Figure 1 *A* is a reproduction of the roentgenogram of the stomach.

11. Pick, L.: Berl. klin. Wchnschr. **49**:16, 1912.

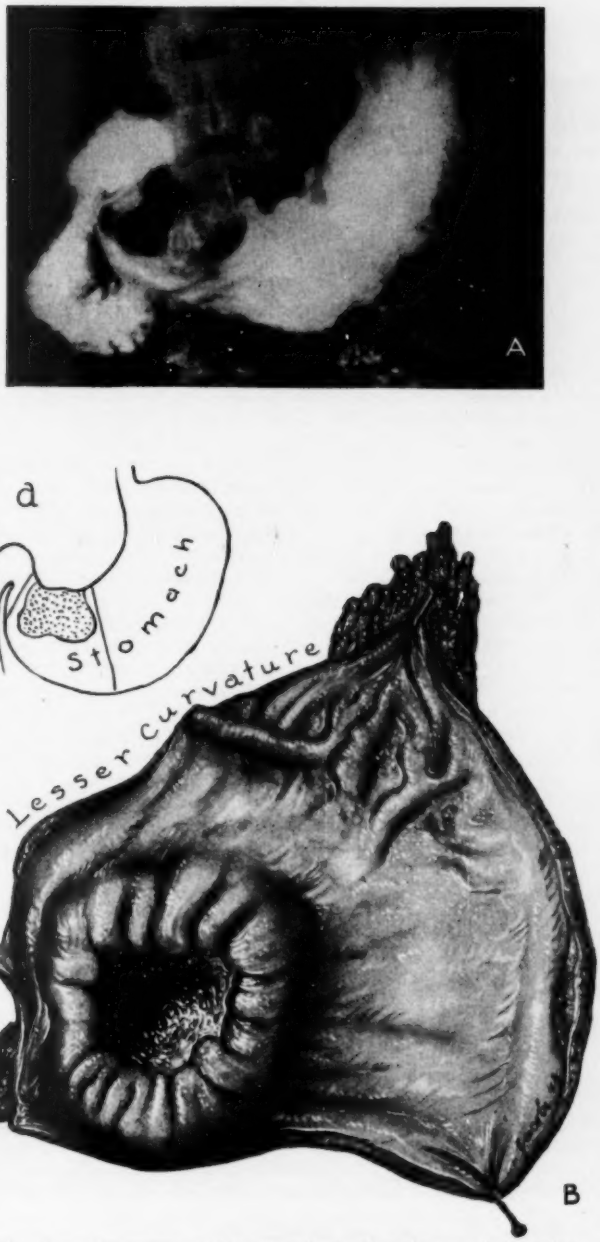


Fig. 1.—*A*, roentgenogram of the stomach showing the prepyloric defect. *B*, drawing of the gross specimen showing the ulcer and indicating (*a*) its prepyloric location.

On November 9 the patient underwent an exploratory laparotomy (Dr. T. B. Jones). The exploration gave negative results except for some rather large succulent lymph nodes extending along the aorta and great vessels. The local lesion was found to involve the prepyloric region of the stomach. The operator described an irregular lobulated elastic tumor invading the superior and posterior aspect of the stomach. It was freely movable and unattached to surrounding structures. The tumor itself could be moved over the underlying serosal layers. No enlarged lymph nodes were noted in the immediate vicinity of the tumor. Resection was decided on and a Billroth anastomosis was done. The postoperative course was uneventful. At the time of writing the patient is asymptomatic and reports a 15 lb. (6.5 Kg.) gain in weight seven months after operation.

The specimen consisted of a 14 by 7 cm. circular segment of stomach. On its posterior aspect the serosa showed a firm, raised, rounded subserosal mass measuring 1.5 by 1 cm. The opened specimen revealed a raised, firm, smooth, oval, sessile excrescence measuring 5 by 3 by 2.5 cm. on the lesser curvature 2 cm. from the distal resection margin. The center of this mass showed a punched-out mucosal ulcer crater measuring 1.5 cm. in diameter and 0.8 cm. in depth. The edges of the ulcer were depressed, and the gastric mucosa was pearl gray (figure 1 *B*). On section the mass revealed pale brown tumor tissue with concentric areas of shiny gray mucoid-like tissue interrupting the structure and extending from submucosa to serosa. Some of these areas gave the impression of small onions in cross section, with circular laminas and easily shelled out central portion. The tumor also appeared in some areas to dissect through the muscle layers in thin sheets. No evidence of encapsulation was found.

Microscopic sections showed gastric mucosa, revealing in one area ulceration with granulation tissue, chronic inflammatory cells, hemorrhage, edema and necrosis, filling in the base of the ulcer. Elsewhere throughout the submucosa and extending through the muscularis to the serosa were sheets of tumor cells, in some areas forming rosettes and in other areas nests and cords (fig. 2 *A*). The cells generally had small round or oval nuclei with discrete nucleoli. The cell cytoplasm was acidophilic and vacuolated. There were a few scattered cells containing large, pleomorphic, hyperchromatic nuclei, some containing multiple nuclei (fig. 2 *B*). Wilder's stain showed an argyrophilic stroma supporting the tumor cells, but no evidence of an intracellular reticulum was demonstrated. Fat stains (sudan IV) showed no intracellular fat. Many of the tumor cells presented faint brown intracytoplasmic stippling after being fixed in chromate solution. Throughout the tumor were spaces containing an acidophilic colloid-like material showing some vacuolation (fig. 2 *C*). Scattered lymphocytes and polymorphonuclear leukocytes were present.

Sections of regional lymph nodes showed no atypical cells.

In reviewing all nonepithelial tumors of the stomach reported in the literature we found 1 case similar to our own. This was reported by Bindslev [12] in 1941, whose report has been translated for us by Dr. Einar Lie. The case is described as one of chromaffin tumor of the lesser omentum invading the stomach wall in a 15 year old youth. The presenting symptoms were severe anemia and melena. The patient was given seven transfusions of whole blood and then submitted to a surgical exploration of the abdomen. Splenectomy and extirpation of the tumor followed. In the lesser omentum and stomach was a 5 by 4 by 4 cm. firm tumor. The tumor lay in the stomach wall, penetrating the mucosa and forming a 1 by 1 cm. funnel-shaped ulcer. The postoperative course was uneventful. The specimen

12. Bindslev, A.: Nord. med. **12**:3472, 1941.

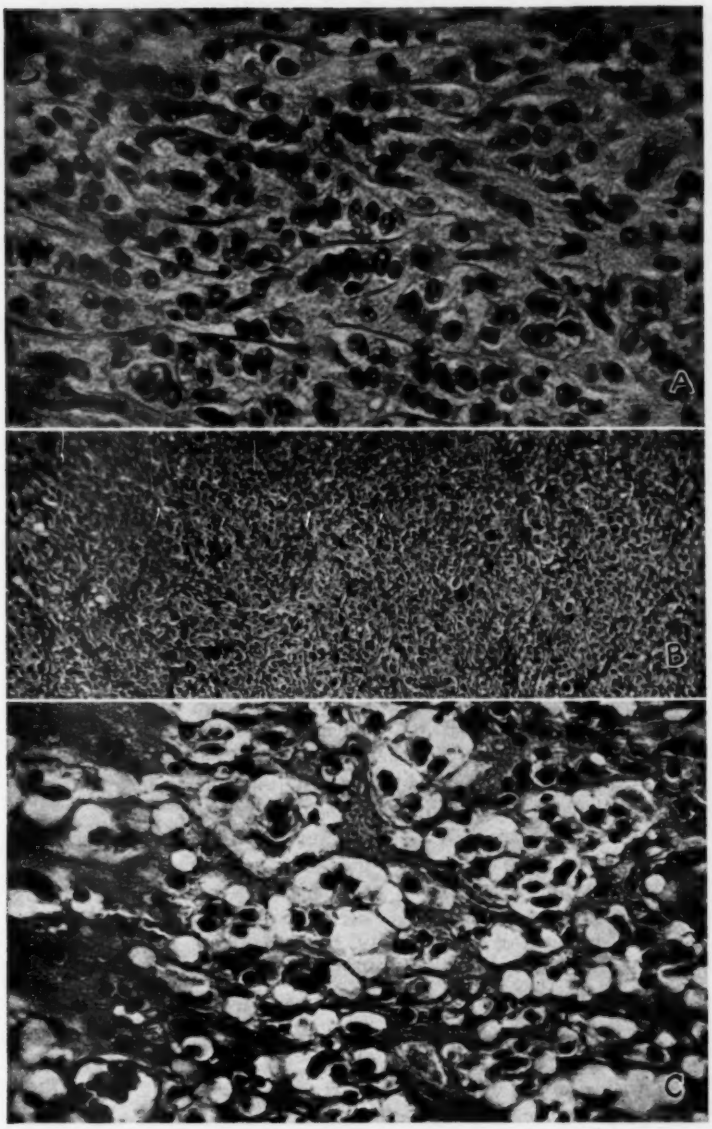


Fig. 2.—*A*, nests and cords of cells in the wall of the stomach. *B*, cellular structure of the tumor. *C*, colloid-like material with vacuolation.

showed a shell of tumor tissue around a central hemorrhagic mass. The tumor cells formed strands and rosettes with some alveolar patterns. The cells were rich in protoplasm with indefinite cell boundaries and round or oval nuclei. Centrally the tissue was necrotic, with lymphocytic infiltrations and old blood pigment. The cells penetrated through the submucosa into the muscularis mucosae. In the submucosa the indefinite cell borders gave the tumor an aspect of cancerous change. The microscopic diagnosis was paraganglioma of the stomach. The spleen showed fibrosis, and a diagnosis of Banti's disease was made.

It is interesting to note that ulceration of the tumor mass associated with hemorrhage was present in both Bindslev's and our own case. Dupuy [13] in 1925 reported a case in which hypertrophy of Auerbach's plexus was found beneath a gastric ulcer. He suggested that the ulcer might have some causal relationship. Masson [14] in 1921 attempted to establish a relationship between proliferation of nerve tissue in the wall of the stomach and gastric ulcers. Canney [15] in 1948 stated that

TABLE 2.—*Intra-Abdominal Neoplasms of Neurogenic Origins (After Ransom and Kay [16])*

1. Nerve sheath tumors
   - A. Benign
     1. Neurilemmoma (schwannoma, perineural fibroblastoma, etc.)
     2. Neurofibroma
     3. Cirsoid or plexiform neurofibroma
     4. Ganglionated neurofibroma
   - B. Cancerous
     1. Neurogenic sarcoma
2. Neuroblastic tumors of sympathetic system
   1. Sympatheticoblastoma
   2. Paraganglioma
   3. Ganglioneuroma

there is little evidence relating proliferated nerve tissue to true benign neurogenic growths.

Recent articles on neurogenic tumors of the stomach follow a classification employed by Ransom and Kay [16] (table 2).

Ransom and Kay [16] in 1940 reported 18 cases of intra-abdominal neurogenic tumors made up of neurilemmoma, neurogenic sarcoma, neurofibroma and ganglionated neurofibroma, and Canney [15] in 1948 reported a case of neurilemmoma and a case of neurofibroma occurring in the stomach. Lockwood [17] in 1932 reported a case of noncancerous neuroblastoma of the stomach. Pitts [18] in 1947 described a case of ganglioneuroma of the stomach. In 1934 and 1937 a sympatheticoblas-

13. Dupuy, M.: Internat. Clin. **4**:164, 1925.
14. Masson, P.: Compt. rend. Acad. d. sc. **173**:262, 1921.
15. Canney, R. L.: Brit. J. Surg. **36**:139, 1948.
16. Ransom, H. K., and Kay, E. B.: Ann. Surg. **112**:700, 1940.
17. Lockwood, B. C.: J. A. M. A. **98**:969, 1932.
18. Pitts, H. H., and Hill, J. E.: Canad. M. A. J. **56**:537, 1947.

toma was reported occurring in the stomach wall in 2 cases by Cieza Rodríguez and Bianchi.[19] The two paragangliomas cited in this paper may now be added to complete the classification as concerns gastric neoplasms.

COMMENT

With the reporting of this case of gastric paraganglioma and the presenting of Bindslev's case in English, attention is directed toward this rare tumor of the stomach and the associated gastric ulcer. These 2 cases provide evidence for the occurrence of the heretofore postulated paraganglioma of the stomach, thereby completing case reports for the classification of Ransom and Kay [16] as regards gastric tumors.

Opportunity is also afforded for some specific comment on the nomenclature of tumors of the chromaffin system. Considerable confusion exists in regard to this group of neoplasms, because classifications by and large rest solely on the morphologic aspects of the tumor cells and do not consider the physiologic possibilities of the new growth. Thus we would agree with Pick [11] and with Belt and Powell,[20] reserving the term "paraganglioma" for extra-adrenal tumors arising from cells of the chromaffin system, with the additional qualification that these extra-adrenal tumors exhibit no hormonal or other specific physiologic activity. Tumors of the adrenal gland, while arising from a similar cell type, are not paragangliomatous in the true sense of the term and should not be classed as paraganglioma. Tumors occurring in extra-adrenal situations which give rise to various clinical symptoms related to hormonal production and secretion should be excluded from a paraganglioma-tous designation, since the cells, while apparently morphologically similar, obviously have other qualities which make them quite different. The biologic behavior of neoplasms is an important factor and must receive consideration together with the histologic information gleaned from microscopic analysis.

SUMMARY

A case of paraganglioma of the wall of the stomach associated with mucosal ulceration and hemorrhage is reported.

A similar case of gastric paraganglioma associated with mucosal ulceration, reported by Bindslev in 1941, is reviewed and summarized in English.

It is suggested that the term "paraganglioma" be reserved for extra-adrenal tumors arising from cells of the chromaffin system and exhibiting no physiologic activity.

19. Cieza Rodríguez, M., and Bianchi, A. E.: Bol. y trab. de la Soc. de cir. de Buenos Aires **18**:1225, 1934. Bianchi, A. E., and Cieza Rodríguez, M.: Novena reunión Soc. argent. de pat. reg. **2**:1018, 1937.

20. Belt, A. E., and Powell, T. O.: Surg., Gynec. & Obst. **59**:9, 1934.

NORMAL OCCURRENCE OF HISTOLOGICALLY DEMONSTRABLE FAT IN THE LIVER OF THE NEWBORN INFANT

JEROME R. DORKIN, M.D.
CAMDEN, N. J.
AND
TOBIAS WEINBERG, M.D.
BALTIMORE

DURING the routine microscopic examination of sections of liver from newborn and stillborn infants on whom autopsies were performed in Sinai Hospital, Baltimore, it was observed that fat occurred in the form of vacuoles in the parenchymal cells of the liver with much more frequency than one might expect on the basis of the associated pathologic processes. It was also observed that there appeared to be a distinct correlation between the amount of fat and the maturity or the degree of prematurity of the infant. Since "fatty change," "fatty infiltration of the liver" and other such diagnoses dependent on the histologic observation of fat in the liver carry a distinctly pathologic connotation, an attempt was made to determine whether or not the fat detected in the livers of newborn and stillborn infants by various staining methods was present normally and whether its presence could be correlated with body weight as an indication of maturity. No record of a similar study could be found in the literature.

MATERIALS AND METHODS

Thirty-one infants, representing all the newborn and stillborn infants subjected to postmortem examination in this hospital between Aug. 18, 1948 and May 8, 1949 whose period of survival was fourteen days or less, were used in this study with the exception of those classified as "foetus sanguinolentus." Portions of liver taken at autopsy were fixed in 4 per cent formaldehyde solution for forty-eight hours. Frozen sections were cut between 15 and 20 microns in thickness. They were stained a few seconds in Harris' hematoxylin and then stained for fat with sudan IV (modification of Herxheimer's solution [1]).[2] Occasionally

From the Division of Pathology, Department of Laboratories, Sinai Hospital, Baltimore 5.

1. The staining solution was made up as follows:

Scarlet red	1.5 Gm.
Alcohol 70 per cent	100 cc.
Acetone, chemically pure	20 cc.

Sections were stained with Harris' hematoxylin for thirty seconds and then transferred to this solution for five minutes. They were then rinsed in 1:1 alcohol-acetone solution and mounted in Apáthy's solution.

2. Cowdry, E. V.: Microscopic Technique in Biology and Medicine, Baltimore, Williams & Wilkins, 1943.

a 1 per cent solution of osmic acid was used, but this did not prove to be as satisfactory. The staining was at times capricious. Often several sections were stained before a particular specimen was accepted as showing or not showing fat. In most instances the presence of stainable fat could be predicted from the appearance of the permanent sections stained by the hematoxylin and eosin technic. In such sections, depending on the amount of fat present, numerous vacuoles could be seen within the liver cells. These vacuoles varied considerably in size, some being small, while others filled almost entire cells, giving them a signet ring appearance. The sudan IV–stained frozen section usually reflected the appearance of the permanent section stained with hematoxylin and eosin.[3] Where the vacuoles were small in the permanent section, the sudanophilic droplets were finely dispersed in the frozen section. Where the majority of the liver cells were replaced by large vacuoles, the sudan IV–stained section would show large orange-red globules. In 1 instance, although the hematoxylin-eosin section had numerous vacuoles within the liver cells, the sudan IV stain revealed none. This case is classified as negative in the series. In all other instances where vacuoles could be seen on hematoxylin and eosin staining, fat could be demonstrated with the sudan IV stain.

Sections were mounted in Apáthy's solution and retained for future study. Within the time limits of the study no sections were observed to fade or otherwise lose their characteristic appearance. The amount of fat present was graded 0 to ++++ as follows:

0—an occasional stained droplet or none at all.
+—minimal: Staining was limited to sudanophilic droplets in an occasional liver cell in most of the lobules.
++—moderate: Sudanophilic droplets were present in several liver cells in each lobule.
+++—marked: Sudanophilic droplets were present in approximately half of the liver cells in each lobule.
++++—very marked: Almost all cells observed contained sudanophilic droplets.

The maturity or prematurity of the infant was estimated most acceptably by the birth weight. Infants weighing 2,500 Gm. and over were considered at term; those weighing less were considered premature. Four weighed less than 1,500 Gm. An attempt to classify the infants with respect to actual and expected dates of delivery proved unsatisfactory because of the errors inherent in calculations of the dates of confinement.

RESULTS

Of the 11 infants weighing 2,500 Gm. or more, 10 had fat in the liver in "marked" to "very marked" amounts (+++ to ++++). The exception was a 2,800 Gm. stillborn infant whose liver showed only moderate amounts of fat (++). In the 1,500 to 2,500 Gm. group, consisting of infants considered to be premature, only 5 of the 16 had more than a minimal amount of fat in the liver. Three of these weighed between 2,400 and 2,500 Gm. and could thus be consid-

3. It is realized that estimates of fat content based on the staining of fat droplets in frozen sections by the sudan IV method are gross approximations. Only neutral fats are demonstrated by sudan IV. Estimates of other lipoid substances require careful chemical analysis and are beyond the scope of this paper. It is hoped that such studies will be undertaken to corroborate the findings presented here.

ered reasonably close to term. The remaining 11 infants in this group and the 4 infants weighing less than 1,500 Gm. had little or no fat in their livers, the amounts being classified 0 to + (table 1). No correlation could be shown

TABLE 1.—*Comparison of Infant Size as Indicated by Weight with Amount of Fat Observed in Liver*

Body Weight, Gm.	Amount of Fat Observed *	Body Weight, Gm.	Amount of Fat Observed *
3,810	++++	2,245	0†
3,407	++++	2,240	+
3,114	+++	2,125	+++
3,070	++++	2,104	0
3,015	+++	2,000	0
3,005	++++	2,045	++
3,000	++++	2,000	0
2,940	+++	1,820	0
2,800	++	1,635	+
2,790	++++	1,561	0
2,715‡	+++	1,525	0
2,497	++	1,230	0
2,485	+++	1,168	+
2,425	++++	815	+
2,320	0	550	0
2,300	+++	.....	..

* 0—none.
+—minimal.
++—moderate.
+++—marked.
++++—very marked.

† The hematoxylin and eosin stain revealed many vacuoles, but these would not stain with sudan IV.

‡ Weights of 2,500 Gm. and over were considered full term; weights under 2,500 Gm. were considered premature.

TABLE 2.—*Distribution of Survival Times in Comparison with Fat Observed in Liver*

Survival Time	0	+	++	+++	++++	Total
Stillborn	2	..	1	..	..	3
Less than 24 hr.	5	2	1	2	1	11
24-48 hr.	1	1	..	1	3	6
48-72 hr.	1	..	..	..	1	2
72-96 hr.	..	..	..	2	..	2
96 hr. and over	2	1	..	2	2	7
						31

TABLE 3.—*Associated Pathological Conditions**

Congenital heart disease	9
Subarachnoid hemorrhage and hemorrhage into the falx	9
Congenital abnormalities of the gastrointestinal tract	5
Bronchopneumonia	5
Congenital hypoplasia of the kidneys	2
Hemopericardium, traumatic (result of intracardiac puncture)	1
Erythroblastosis fetalis	2
Prematurity †	8

* More than one diagnosis is listed for each case.

† This was the primary diagnosis in instances in which no other more definitive morbid condition could be found.

between the amount of fat in the liver and the survival time (table 2). There was no definite correlation with the associated pathologic changes (table 3).

COMMENT

This study shows that the presence of fat in the liver of newborn, full term infants is probably normal. Heretofore, it has usually been considered abnormal and has given rise to the diagnoses of "fatty change of the liver" and "fatty infiltration of the liver." Often the fat has been explained on the basis of anoxemia, but this can scarcely be expected to produce such an extensive distribution of fat within the short survival time of some of the infants studied. Of interest also is the fact that the more premature the infant the less the fat that can be demonstrated in its liver. The following arguments are offered in an attempt to show that histologically demonstrable fat in the liver is part of the normal physiologic and metabolic background of the newborn infant.

Boyd and Wilson,[4] calculating the concentrations of lipoid materials in the cord blood of human subjects, were able to show that umbilical vein blood leaving the placenta and supplying the fetus contained higher concentrations of certain lipoid materials than umbilical artery blood returning to the placenta. They concluded that certain lipoid substances are added to umbilical blood by the placenta and are removed or absorbed by the fetus. While studies are not available showing the rate at which fats are stored in the human fetus, Imrie and Graham,[5] studying the fat content of the embryonic guinea pig liver, showed quite definitely that fat accumulates in the livers of guinea pigs during the latter part of gestation. No evidence of this accumulation was found until the embryos attained a weight of about 40 Gm. However, as their weights increased above this value, the increase in fat was quite impressive. The rapid disappearance of the fat after birth "suggests that it (fat) is material required by the young animal after its communication with the maternal circulation has ceased."

Many interesting studies have been made in an attempt to calculate the relative amounts of fat and carbohydrate metabolized by the newborn infant. Needham[6] stated that in utero the respiratory quotient is close to 1.0, indicating that carbohydrate forms almost the only source of energy. Benedict and Talbot[7] found the respiratory quotient in the neighborhood of 0.90 shortly after birth, reflecting the utilization of the stored glycogen available at birth. The amount of stored carbohydrate is apparently limited, however, and over the subsequent seventy-two

4. Boyd, E. M., and Wilson, K. M.: J. Clin. Investigation **14**:7, 1935.

5. Imrie, C. G., and Graham, S. G.: J. Biol. Chem. **44**:243, 1920.

6. Needham, J.: Chemical Embryology, New York, The Macmillan Company, 1931, vol. 2.

7. Benedict, F. G., and Talbot, F. B.: The Physiology of the Newborn Infant: Character and Amount of Katabolism, no. 233, Washington, Carnegie Institute, 1915.

hours the average respiratory quotient tends to fall, reaching its lowest value of 0.73 on the third day. This would correspond to a metabolic mixture of approximately 90 per cent fat. By the end of the first week the average respiratory quotient rises to about 0.80 and metabolic mixtures consisting of approximately one-third carbohydrate and two-thirds fat are being used. Once milk can be utilized in adequate amounts, the utilization of carbohydrate increases. These results indicate the importance of fat in the metabolism of the newborn infant, perhaps best summarized in this statement by Smith[8]:

> . . . The fetus probably uses little fat for its heat production but stores a considerable amount against later emergencies; how this is brought across the placenta is not known. Once the newborn infant has fairly well depleted its available glycogen stores (which is usually within a few hours after birth) it begins to depend largely upon reserves of fat for energy. During this process there is a greatly increased transport of fat substances in the blood, an increase not solely due to the newly introduced element of absorption from the alimentary tract.

Two statements, then, can be made with regard to the normal presence of considerable quantities of histologically demonstrable fat in the liver of the newborn, full term infant:

1. In the interval between the rapid exhaustion of available carbohydrate stores and the assimilation of adequate dietary carbohydrate the fat stores in the full term infant provide a suitable, plentiful and potent source of energy.

2. Ample opportunity for such storing of lipoid materials in the liver occurs in utero through placental transport.

The noticeable lack of fat stores in the liver of a premature infant is probably evidence that hepatic fat storage is a physiologic preparation for birth. From a study of the estimated dates of confinement of the mothers of infants in this series there is every indication that the storing of fat in the liver apparently begins in earnest during the last month of intrauterine life.

SUMMARY

Fat which can be histologically demonstrated by certain staining methods in the livers of newborn, full term infants is probably a normal finding.

By the use of the same methods it has been shown that premature infants do not have appreciable quantities of fat in their livers.

A possible physiologic explanation is offered for the normal presence of histologically demonstrable fat in the liver of the newborn infant at term and its absence in the premature infant.

8. Smith, C. A.: The Physiology of the Newborn Infant, Springfield, Ill., Charles C Thomas, Publisher, 1946.

DEGENERATIVE RENAL LESIONS INDUCED BY PROLONGED CHOLINE DEFICIENCY

J. J. LALICH, M.D.
B. E. KLINE, M.S.
AND
H. P. RUSCH, M.D.
MADISON, WIS.

ALTHOUGH most studies on choline deficiency have been concerned with changes in the liver, a number of investigators have also observed the effect of this deficiency on the kidney. Hemorrhagic lesions of the kidneys of rats have been described repeatedly [1] since the initial observations of Griffith and Wade.[2] In acute (less than six months) choline deficiency Gyorgy and Goldblatt [1c] observed that the initial lesions were in the tubules, with glomeruli affected only in the terminal stages. Engel and Salmon [1b] observed minimal thickening of the renal capsule with some connective tissue proliferation in the healing phase in rats subjected to acute choline deficiency. Christensen observed a rather remarkable degree of healing four to sixteen months after acute periods of choline deficiency.[1a] In the healing phase he observed wedge-shaped areas of cortical atrophy, calcified tubular remnants and disappearance of eosin casts with persistence of dilated atrophic tubules.

Hartroft and Best [3] succeeded in producing hypertension and renal changes in some of their rats when choline-deficient diets were maintained for periods longer than six months. After comprehensive study of the pathogenesis of the acute "hemorrhagic renal syndrome," Hartroft pointed out that the sudanophilic material accumulating in the proximal convoluted tubules was related to the subsequent cortical capil-

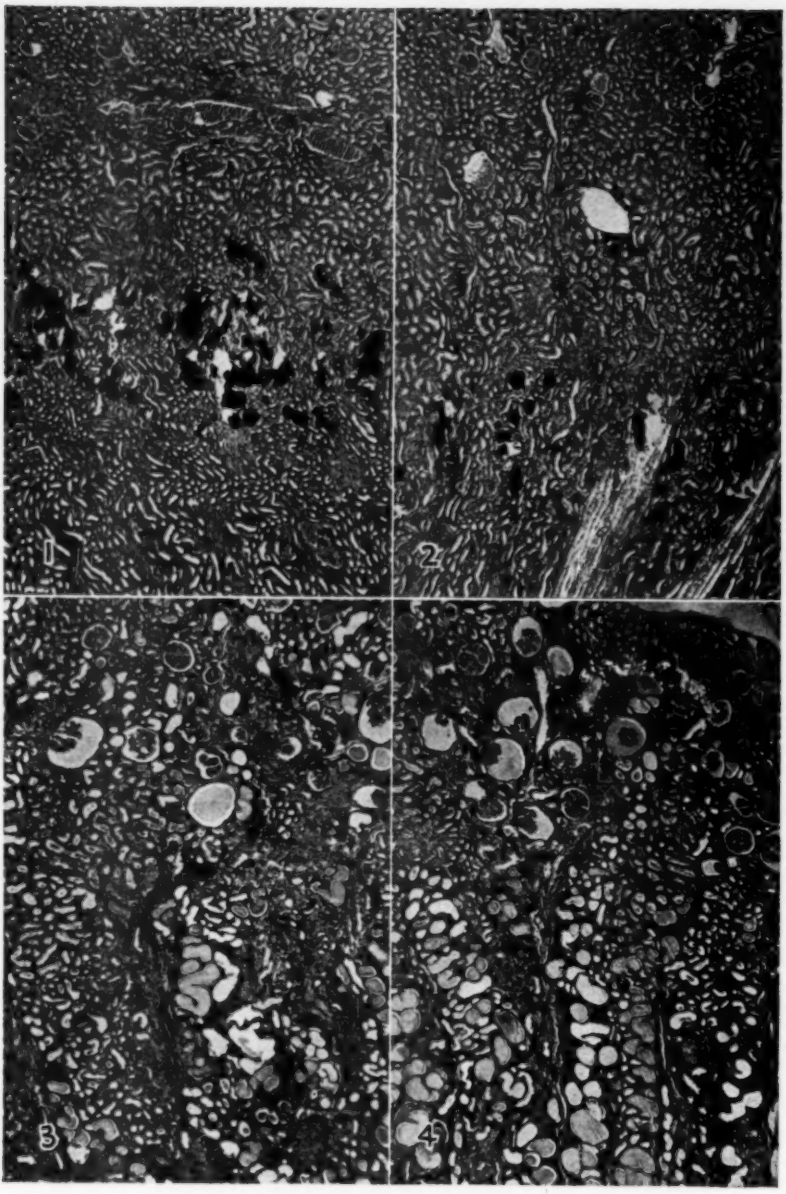
This investigation was aided by the Jonathan Bowman Fund for Cancer Research.

From the Department of Pathology and McArdle Memorial Laboratory, University of Wisconsin Medical School.

1. (*a*) Christensen, K.: Arch. Path. **341**:633, 1942. (*b*) Engel, R. W., and Salmon, W. D.: J. Nutrition **22**:109, 1941. (*c*) Gyorgy, P., and Goldblatt, H.: J. Exper. Med. **72**:1, 1946. (*d*) Hartroft, W. S.: Brit. J. Exper. Path. **6**:483, 1948.

2. Griffith, W. H., and Wade, N. J.: (*a*) J. Biol. Chem. **131**:567, 1939. (*b*) **132**:627, 1940.

3. Hartroft, W. S., and Best, C. H.: Brit. M. J. **1**:424, 1949.



Figures 1-4

(See legends on opposite page)

lary ischemia which induced the necrosis of tubular epithelium. In chronic experiments with 150 Gm. rats, however, sudanophilic droplets were not so numerous and renal cortical ischemia and focal necrosis of the epithelium did not occur.[1d] It would appear, therefore, that other factors are responsible for the renal destruction which may be produced by chronic (six months or longer) choline deficiency in weanling rats.

Copeland and Salmon [4] reported the production of neoplasms in rats fed choline-deficient diets from eight to sixteen months. Erickson and Goebbel [5] corroborated this observation in a smaller percentage of their animals and also observed numerous collections of eosinophilic casts with tubular dilatation and fibrosis in the kidneys of a majority of their rats. Since their interest was primarily in the influence of chronic choline deficiency on tumor production, the renal changes were not described in detail in their preliminary report. The present paper describes the extensive alterations of the renal parenchyma as they appeared at intervals in animals subjected to a choline deficiency for many months.

METHOD

Hooded rats were obtained from the Laboratory of Animal Nutrition, Agricultural Experiment Station, Alabama Polytechnic Institute, Auburn, Ala; Sprague-Dawley rats were secured from the Holtzman Laboratory Animals, Incorporated,

4. Copeland, D. H., and Salmon, W. D.: Am. J. Path. **22**:1059, 1946.
5. Erickson, C. C., and Goebbel, W.: Federation Proc. **8**:354, 1949.

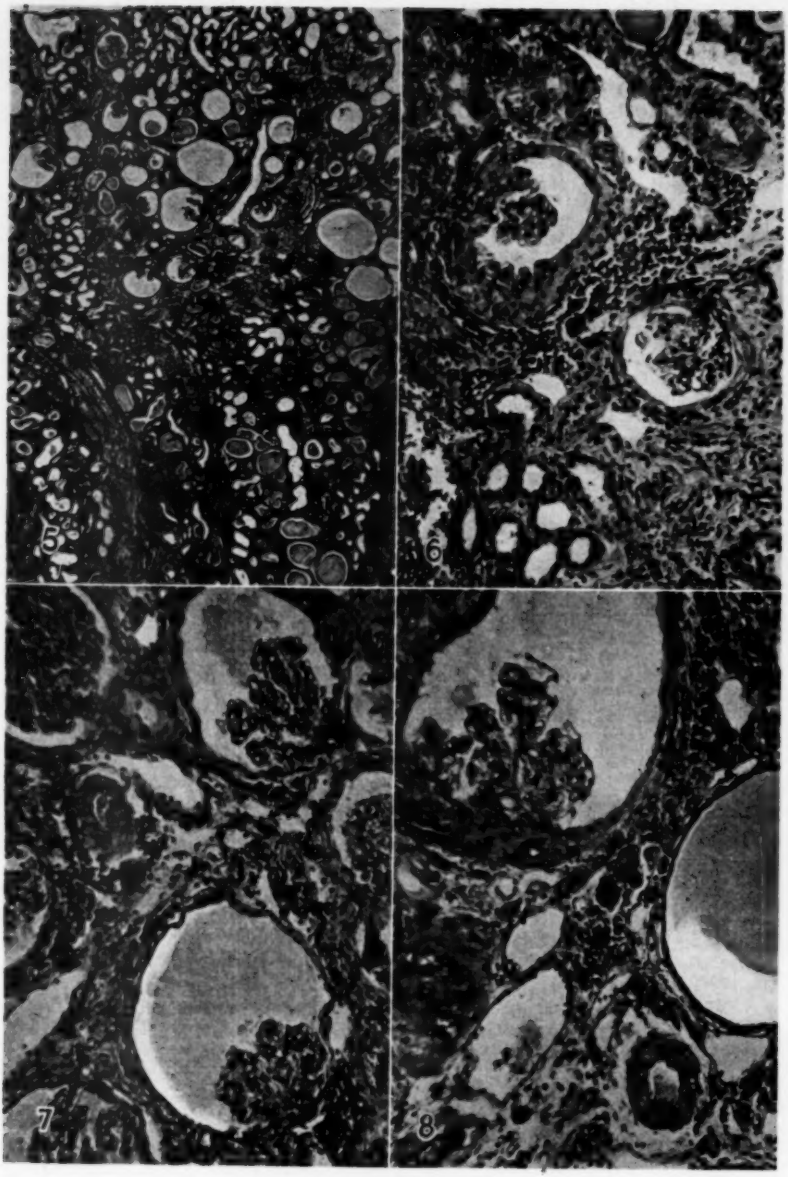
Explanation of Figs. 1-4

Fig. 1.—Control rat 2 was fed the control diet for ten months. There is calcification at the corticomedullary junction. The glomeruli and the tubules are normal. The lumens of the tubules are empty. Hematoxylin and eosin stain; × 25.

Fig. 2.—Control rat 7 was fed the control diet for fourteen months. The glomeruli and the tubules were considered normal. There is an area of calcification, which is limited principally to the corticomedullary zone. There is an eosinophilic granular material in some tubules; however, tubular dilatation was not observed. Hematoxylin and eosin stain; × 25.

Fig. 3.—Test rat 10 was maintained on a choline-deficient diet for seven and a half months. There was 2 plus destruction of the cortical elements. In this field there are collections of acidophilic casts and tubular dilatation with atrophy of epithelial elements. Bowman's spaces are dilated; some of these contain eosinophilic material. Note the compression of the glomerular tufts where eosinophilic material fills Bowman's spaces. In this field there are also normal-appearing glomeruli. Fibrosis and lymphocytic infiltration are most pronounced near the corticomedullary junction. Only a few of the tubules have undergone calcification in this field. Hematoxylin and eosin stain; × 25.

Fig. 4.—Test rat 20 was on a choline-deficient diet for thirteen months. The cortical destruction was considered 2 plus. In the section shown the cast collection and subsequent tubular dilatation are pronounced. Compression of glomerular tufts is focal in nature, and it is associated with swelling of Bowman's capsules and the presence of eosinophilic material in Bowman's spaces. There is minimal calcification. The interstitial fibrosis and lymphocytic infiltration are not as prominent as in the previous figure. Hematoxylin and eosin stain; × 25.



Figures 5-8

(See legends on opposite page)

of Madison, Wis. These animals were bred in the McArdle Memorial Laboratory for Cancer Research of the University of Wisconsin and were fed the same stock diet as was used by Copeland and Salmon.[4] The stock diet contained: ground wheat, 60.5; meat scraps, 9.4; alfalfa leaf meal, 1.9; iodized salt, 0.5; black strap molasses, 4.7; whole milk powder, 5; crude casein, 12; lard, 5, and cod liver oil, 1 per cent. The experimental diet which was used in this experiment was similar to 43A of Copeland and Salmon.[4] The control rats received diet 43A with 2 Gm. of choline added per kilogram of diet.

Rats of both strains were weaned at 23 or 24 days of age. Approximately two thirds of them were fed the low choline and one third the added choline diet. All of the choline-deficient rats were given daily supplements of choline in water by stomach tube during the first week or two to get them over that critical period when they had a great need for choline. The Sprague-Dawley rats were usually given 2 to 4 mg. doses and the Alabama rats 4 to 6 mg. By the end of the second week the rats usually gained weight on the diet without choline supplementation. However, they were weighed daily throughout the critical period of the experiment and were given choline whenever a sharp drop in weight indicated the need. Older rats were given 20 to 40 mg. doses at such times. When the animals failed to respond to one or two doses of choline supplement, their condition deteriorated rapidly and they died.

Test animals were selected for microscopic study from rats which died or from those which were killed when they became inactive or lost weight rapidly and stopped eating. The majority of the control rats had to be killed, since only a few died with bronchopneumonia. The tissues were fixed in 10 per cent formaldehyde solution and stained with hematoxylin and eosin. A total of 15 control and 23 test rats subjected to choline-deficient diets for periods of six to nineteen months are included in the present study.

RESULTS

Gross Observations.—The significant pathologic alterations occurred in the livers, the kidneys and the lungs. Fatty livers with cirrhotic

---

EXPLANATION OF FIGS. 5-8

Fig. 5.—Test rat 18 was maintained on a choline-deficient diet for twelve months. The tubular and glomerular degeneration was considered to be 3 plus. In this section there are interstitial fibrosis, lymphocytic infiltration and focal calcification. The remaining tubules are clearly dilated and contain eosinophilic casts. The glomerular alterations are advanced; there is compression of tufts; some are small, avascular and cellular, whereas other glomeruli have completely disappeared. Hematoxylin and eosin stain; × 25.

Fig. 6.—Section of a kidney of the same animal as that in figure 3. There is thickening of Bowman's capsule with destruction of glomeruli. The tubules are replaced by fibrosis and lymphocytic infiltration. Hematoxylin and eosin stain; × 150.

Fig. 7.—Test rat 14 was on a choline-deficient diet for nine months. The cortical destruction was considered to be 2 plus. Note the definite fibrosis of Bowman's capsules, the eosinophilic material in Bowman's spaces and the variable alterations in the glomeruli. There are also loss of tubules, interstitial fibrosis, lymphocytic infiltration and arteriolar hypertrophy. Hematoxylin and eosin stain; × 150.

Fig. 8.—Test rat 21 was on a choline-deficient diet for fourteen months. The degeneration of tubules and glomeruli was 3 plus. Note the advanced tubular and glomerular destruction. Fibrosis, lymphocytic infiltration, arteriolar hypertrophy and calcification are also evident. Hematoxylin and eosin stain; × 150.

nodules were observed in the choline-deficient rats. In the control rats almost all of the livers and kidneys were normal on gross inspection. In the deficient group, depending on the severity of renal involvement, there was a progressive increase in size of the kidneys to approximately two times normal. In addition, a fine granularity of the surface with or without retention cysts, was also apparent. The majority of the rats in the control and deficient groups had purulent bronchitis and terminal bronchopneumonia. In over half of these animals these conditions were associated with severe chronic bronchiectasis, cystic dilatation of bronchi and multiple pulmonary abscesses.

Microscopic Observations.—Before an analysis of the microscopic pathologic changes is attempted, a description of the progressive tendency of the renal lesions may prove helpful. The most common early observation was the presence of eosinophilic or slightly basophilic staining casts of a homogeneous character. The number as well as the size of these casts varied considerably. Sometimes desquamated epithelial cells made up a part of the cast; these, however, were never a conspicuous feature. Initially there were numerous small casts principally in the distal convoluted tubules and Henle's loops with no or slight evidence of dilatation. Later the casts were five to ten times larger, less numerous, but associated with distinct tubular dilatation. The epithelial cells of the dilated tubules were flattened and atrophic or absent. Initially the tubular obstruction occurred in segments with intervening uninvolved parenchyma, in which the epithelial cells might be swollen and vacuolated. Still later, depending on the extent of the tubular obstruction, the pathologic change became generalized. In such cases there was a reduction in the number of tubular elements, and the remaining tubules were surrounded by connective tissue and foci of lymphocytes. In these sections only isolated granules of hemoglobin pigment were to be observed, either in the epithelial cells of the convoluted tubules or in the phagocytes which had invaded the interstitial space.

The degeneration of the glomerular tufts appeared to be secondary to the tubular obstruction, since most of the compressed tufts occurred in areas of tubular obstruction. Associated with the shrinking of the glomerular tuft there was an extensively dilated Bowman space filled with eosinophilic material. Normal glomeruli and tubules were observed in areas adjoining those just described. Bowman's spaces in which there were no glomerular tufts or in which the glomeruli were undergoing fibrosis were found most frequently in areas of diffuse tubular obstruction and extensive interstitial fibrosis. At this late stage hyalinization and thickening of Bowman's capsule were also a common finding.

Alterations of renal arteries were neither so prominent nor so common as the tubular and glomerular destruction. Intimal calcification

of the larger renal arteries near the hilus was seen infrequently. Hyalinization and hypertrophy of arterioles were observed in over half of the choline-deficient rats. The arteriolar changes, however, were focal in distribution and were present only in areas of glomerular and tubular destruction. The interstitial fibrosis and the lymphocytic infiltration were roughly equivalent to the tubular and glomerular changes. Significant interstitial fibrosis occurred only in the deficient rats; it was not observed in the control group.

Calcification occurred principally in the area of the corticomedullary junction. The calcification appeared to be intratubular and was present in over 90 per cent of the animals. It appeared to be of similar severity and frequency in the test and control groups. Calcification is unusual in control animals, and the cause for it is not evident.

The microscopic data concerning 7 control and 14 test Alabama hooded rats has been assembled in the accompanying table to illustrate more fully the influence of chronic choline deficiency on the renal parenchyma. The extent of involvement was based on the percentage of tubules and glomeruli affected in one section. On this basis the sections examined were separated into four groups. Sections were considered normal when less than 5 per cent of the tubules contained casts and there was no evidence of glomerular destruction. One plus indicates 5 to 20 per cent; 2 plus, 20 to 50 per cent, and 3 plus, 50 per cent or more involvement of the tubular and glomerular elements. The arterial changes, the interstitial fibrosis and the calcification are indicated as being present or absent by a plus or a minus sign.

Examination of the table shows that 1 of 7 control rats exhibited a significant cast accumulation with some glomerular destruction. In the remaining 6 the kidneys were normal except for the presence of calcification in 3. In all of the 14 Alabama hooded rats restricted to a choline-deficient diet for periods of six to fourteen months renal changes were observed. There was 1 plus involvement in 5, 2 plus in 7 and 3 plus in 2. Interstitial fibrosis was not conspicuous until 20 to 50 per cent of the tubules were involved. Arteriolar hyalinization and hypertrophy were more apparent in kidneys with greater destruction.

Observations in both control and deficient Sprague-Dawley rats were in general agreement with those in the Alabama strain. Of 8 control rats maintained eleven to nineteen months, 5 had normal kidneys. There was 1 plus tubular involvement without significant glomerular destruction in 3. Of 9 deficient rats maintained from ten to eighteen months, 3 had normal kidneys. There was 2 plus alteration of tubules and glomeruli in 4 and 1 plus in 2. The microscopic observations suggested that the renal changes were more difficult to produce in the Sprague-Dawley strain.

Since the influence of choline deficiency on the liver has been repeatedly described,[6] only the frequency of such changes will be given. A diffuse fatty infiltration associated with regenerating liver and discontinuous periportal fibrosis was observed in 18 of 23 test rats and in none of the controls. Confluent periportal fibrosis with greater collections of connective tissue was observed in 12 of 23 rats. Normal livers were found in 16 control and 4 test rats.

Sections of lungs from 30 rats were examined. Moderate to severe bronchiectasis associated with terminal bronchopneumonia occurred in 16 test and 7 control rats. In 7 control rats the lungs were considered normal.

The Effect of Chronic Choline Deficiency on the Kidneys of Alabama Rats

				Renal Alterations Observed†				
Rat	Diet*	Sex	Time, Mo.	Casts and Tubular Obstruction	Glomerular Destruction	Interstitial Fibrosis	Arterial Degeneration	Calcification
1	C	F	7.5	—	—	—	—	+
2	C	F	10.0	—	—	—	—	+
3	C	M	11.0	—	—	—	—	—
4	C	F	12.0	—	—	—	—	—
5	C	M	12.5	2+	1+	—	—	+
6	C	M	14.0	—	—	—	+	+
7	C	F	14.0	—	—	—	—	—
8	T	M	6.0	1+	—	—	—	+
9	T	M	7.0	1+	—	—	—	+
10	T	M	7.5	2+	2+	+	+	+
11	T	F	7.5	2+	2+	+	+	+
12	T	M	8.0	1+	1+	—	—	—
13	T	M	8.5	1+	1+	—	+	+
14	T	M	9.0	2+	2+	+	—	+
15	T	F	9.5	1+	1+	—	—	+
16	T	M	10.0	2+	3+	+	+	+
17	T	F	11.5	2+	2+	+	—	+
18	T	M	12.0	3+	3+	+	+	+
19	T	F	12.0	3+	2+	+	+	+
20	T	M	13.0	2+	2+	+	+	+
21	T	F	14.0	3+	3+	+	+	+

* C is the control diet; T, the choline-deficient diet.
† In the first two columns 1+ indicates that 5 to 20 per cent of the tubules and glomeruli were affected in one section; 2+, 20 to 50 per cent; 3+, 50 per cent or more. In the last three columns + or — signifies present or absent as the case may be.

COMMENT

The magnitude of renal destruction observed in this study is in agreement with that observed by Erickson and Goebbel.[5] The lesions described are more extensive than those observed by Hartroft [1d] and Hartroft and Best.[3] This is probably due to the fact that weanling rats were used and the time interval was usually longer in our studies. Vacuolation of epithelial cells of the proximal convoluted tubules, such as is described by Hartroft, was observed in all of the test animals. Recent hemorrhage was not observed in the series. Evidence of previous

6. Engel and Salmon,[1b] Griffith and Wade.[2]

hemorrhages as judged by the presence of hemosiderin in the interstitial tissue was minimal or absent.

The renal destruction appeared to be associated with and to follow the accumulation of eosinophilic and basophilic casts in the distal convoluted tubules and Henle's loops. Whether the casts were due to alteration of glomerular permeability with loss of plasma proteins or were associated with elimination of other constituents of plasma is not known, although the former hypothesis seems more plausible. The presence of numerous casts with tubular and glomerular destruction of a segmental character suggests a degenerative rather than an inflammatory lesion. This opinion was further enhanced by a failure to observe neutrophilic cells in the renal pelvis, the collecting tubules or the interstitial tissue of the medulla. In view of the aforementioned findings, the focal collections of lymphocytes in the interstitial spaces were thought to be a sequel of the destruction of tubular and glomerular elements rather than a manifestation of primary chronic inflammatory reaction.

On the basis of our observations it appears that the probable sequence of events was as follows. The initial lesion was vacuolation of epithelial cells of the proximal convoluted tubules. This was followed by the collection of eosinophilic and basophilic casts in the distal convoluted tubules and Henle's loops. The casts eventually assumed such proportions as to cause obstruction of the proximal segment with tubular dilatation. At this stage the tubules resembled thyroid acini, as pointed out by Erickson.[7] Next, eosinophilic proteinaceous material collected in Bowman's space, dilating the capsule and compressing the glomerular tufts. It is quite possible that protracted intratubular obstruction may be responsible for the hyalinization and fibrosis of Bowman's capsule. Compression of the tufts, thickening of the basement membrane and fibrosis or atrophy and disappearance of glomeruli, leaving empty Bowman's spaces, were observed. The glomerular involvement was so extensive that over 95 per cent exhibited some degree of change. Following the glomerular alterations, focal hyalinization and hypertrophy of arterioles occasionally became evident in the adjacent area.

It seems doubtful whether the present observations have any direct bearing on the pathology of renal disease in man. The observed changes, however, are of sufficient magnitude to account for the hypertension which was first described by Hartroft and Best.[8] The renal destruction is probably responsible for losses of substantial quantities of protein in the urine. In these rats, moreover, a profound anemia develops and they apparently become increasingly susceptible to pulmonary infection. For these reasons, then, in future experiments on chronic choline

7. Erickson, C. C.: Personal communication to authors.

deficiency it must be borne in mind that the renal changes which occur may be responsible for some of the extrarenal changes which are observed in the animals.

SUMMARY

A chronic choline deficiency was maintained for as long as six to nineteen months in rats of the Alabama and Sprague-Dawley strains. Twenty-three choline-deficient and 15 control rats that survived six months or longer were studied. The renal alterations were severe: From 20 to more than 50 per cent of the renal parenchyma was destroyed in 13 of 23 deficient rats, whereas only minimal and insignificant changes were observed in the control group. The renal destruction appeared to be associated with and secondary to the accumulation of eosinophilic and basophilic casts which took place in the distal convoluted tubules and in Henle's loops.

Notes and News

Legal Medicine Program Established in Louisiana.—At Tulane University of Louisiana, New Orleans, an effective integration of the fields of law and medicine is under way on the graduate and undergraduate levels. Dr. Hubert Winston Smith, former professor of legal medicine at the University of Illinois, Chicago, has been named research professor of law and medicine. He will serve also as professor of law and professor of legal medicine in the college of law and the school of medicine, respectively. New courses and seminars will be devoted to more complete training of trial lawyers in the science of proof and of handling personal injury litigation and in legal psychiatry. Long range plans call for projected developments in clinical legal medicine, legal pathology and scientific crime prevention and detection. A course in legal medicine has been established. It is planned also to offer a course in the medicolegal aspects of behavior problems.

During the past few years Dr. Smith has joined with other specialists in research aimed at reformulating medical sciences in terms of their legal applications and utility. The result has been that more than one hundred and twenty-five studies have appeared in both medical and legal journals. During World War II Dr. Smith served as officer in charge of the legal medicine branch of the Bureau of Medicine and Surgery, United States Navy.

George Minot Lectureship Established.—Officers of the Section on Experimental Medicine and Therapeutics of the American Medical Association, desiring to honor, by establishing a "name lecture," an American investigator who has made an exceptional contribution to the development of clinical investigation and therapeutics, suggested at the Interim Session of the Association in St. Louis last November that immediate steps be taken to obtain approval of the idea from the Council on Scientific Assembly and that the lecture be named in honor of Dr. George Richard Minot, Boston, whose contributions to medical knowledge of the causes and methods of control of pernicious anemia have been recognized throughout the world. Both suggestions were unanimously supported by the executive committee of the Section, and approval has been obtained from the Council on Scientific Assembly. A committee of former chairmen of the Section—Drs. Edgar van Nuys Allen, Rochester, Minn.; Walter Bauer, Boston, and Carl Dragstedt, Chicago—has been appointed to work out details. The first lecture will be given either at the 1950 or at the 1951 meeting.

Appointments.—R. S. Fisher, resident fellow in legal medicine at Harvard Medical School, has been appointed assistant professor of pathology in the medical faculty of Western Reserve University, Cleveland.

Alvan G. Foraker and C. Merrill Whorton have been appointed assistant professors in the department of pathology at Emory University School of Medicine, Atlanta, Ga.

F. W. Sunderman, formerly head of the department of clinical pathology at the Cleveland Clinic Foundation, is now at the head of the department of clinical pathology in the University of Texas Postgraduate School of Medicine.

Philip Hench, of the Mayo Clinic, has been appointed chairman of the Arthritis and Rheumatism Study Section of the United States Public Health Service, National Institutes of Health. The other members are Walter Bauer, of Boston; Granville A. Bennett, of the University of Illinois School of Medicine; Jerome W. Conn, of the University of Michigan; W. Paul Holbrook, of Tucson, Ariz.; Robert Loeb, of New York; F. J. Moore, of the University of Southern California School of Medicine; Jane A. Russell, of Yale University School of Medicine; Emil L.

Smith, of the University of Utah School of Medicine, and Alfred L. Wilds, University of Wisconsin.

William McDowell Hammon, assistant director of the Hooper Foundation, University of California, has been appointed professor and head of the department of epidemiology at the University of Pittsburgh Graduate School of Public Health. In this position Dr. Hammon will also be responsible for the instructional and research interests of the school in the field of microbiology. His appointment is effective Feb. 1, 1950.

New York Medical College has promoted Francis D. Speer, assistant professor of pathology to professor of pathology and clinical pathologist and director of the department. He succeeds George K. Higgins, who has resigned.

Béla Halpert, formerly director of laboratories at the University of Oklahoma Hospitals and professor of clinical pathology in the University of Oklahoma School of Medicine, has been appointed chief of laboratory service at the Veterans Administration Hospital at Houston, Texas, and associate professor of pathology at Baylor University College of Medicine, effective Oct. 1, 1949.

Fund for Research in the Field of Lymphatic Leukemia.—The National Research Council announces that the estate of Charles R. Blakely has donated $25,000 for the support of research bearing on lymphatic leukemia. Applications for grants are now being entertained. Further information and application forms may be secured from the Chairman of the Division of Medical Sciences, National Research Council, 2101 Constitution Avenue, N. W., Washington 25, D. C.

Fellowships.—The National Research Council of Canada will offer thirty-one postdoctorate fellowships for the year 1950-1951, eighteen of which will be awarded in chemistry, three in atomic energy research and ten in physics. The stipend of $2,820 per annum is supplemented by travel grants to successful candidates from abroad. While appointments in the atomic energy project are restricted to Canadian citizens and British subjects, applicants of all nationalities are welcome in the chemistry and physics divisions. Application forms and further information may be obtained from The Secretary, Laboratories Awards Committee, National Research Council, Ottawa. Applications should be received not later than Feb. 15, 1950.

Donald B. McMullen, associate professor of preventive medicine and public health, University of Oklahoma Medical School, has returned after twenty-six months' leave of absence. Dr. McMullen has been working on the epidemiology and control of schistosomiasis japonica in Tokyo, where he has been senior parasitologist with the 406th Medical General Laboratory.

Deaths.—Ralph R. Parker, 61, entomologist, died of a heart attack at his home in Hamilton, Mont., September 4. Director of the Rocky Mountain Laboratory of the National Institute of Health, Dr. Parker was co-discoverer of a vaccine for Rocky Mountain spotted fever.

Dr. William R. Bonnelle, of Fort Wayne, Ind., died May 26, aged 75, of arteriosclerotic heart disease and diabetes mellitus. Dr. Bonnelle was a charter member of the American Society of Clinical Pathologists, one of the founders of the Indiana State Pathological Association, member of the College of American Pathologists, American Association for the Advancement of Science, American Heart Association and Fort Wayne Academy of Science. He served in France and Germany during World War I and was a member of the Indiana Medical Advisory Board no. 4, Selective Service System in World War II.

Dr. Giulio Andrea Pari, who occupied the chair of medical pathology in Padua University, Italy, since 1925, has died at the age of 69. He was a productive investigator, especially in the physiology of neural centers.

Samuel Harold Gray, St. Louis, died in Cameron, Wis., August 18, aged 52. Dr. Gray was associate professor of pathology at Washington University School of Medicine. He was a specialist certified by the American Board of Pathology and a member of the American Association of Pathologists and Bacteriologists, the College of American Pathologists, the American Society for Experimental Pathology and the American Society of Clinical Pathologists. He was affiliated with the Jewish Hospital and served during World War II.

John Joseph Larkin Jr., Boston, died in Sterling, Mass., August 26, aged 35, of accidental drowning. He was a member of the American Medical Association and the College of American Pathologists; a specialist certified by the American Board of Pathology; assistant professor of pathology at Tufts College Medical School; pathologist at the Holy Ghost Hospital in Cambridge, where he was in charge of cancer research, and St. Elizabeth's Hospital. He served in World War II from 1942 to 1945 as captain, later major, at Base Hospital 136 in England and France.

William Henry Watters, 73, pathologist, died October 10 in Hyannis, Mass. Dr. Watters was at one time professor of pathology at Boston University and for many years had been an associate in the legal department of the Harvard University Medical School.

Society News.—At the meeting of the Wisconsin Society of Pathologists in Milwaukee on October 5, the following officers were elected for the ensuing year: president, W. A. D. Anderson, Milwaukee; vice president, Walter H. Jaeschke, Madison; secretary-treasurer, Robert S. Haukohl, Milwaukee, and board of censors, J. B. Miale, Marshfield.

Awards.—At the annual meeting of the American Cancer Society, Bowman C. Crowell, Chicago, was awarded the society's 1949 medal "in recognition of his outstanding contributions to the control of cancer." Clifford Calvin Nesselrode, retiring president of the society who presented the award, praised Dr. Crowell's "signal accomplishments in developing and extending improved services to cancer patients through diagnostic and treatment clinics." Dr. Crowell recently retired from his position as associate director of the American College of Surgeons.

Cancer Research Fellowshps.—The British-American Exchange Fellowships in Cancer Research, awarded by the American Cancer Society on the recommendation of the Committee on Growth of the National Research Council, are now open to citizens of the United States who have M.D., S.D. or Ph.D. degrees. Fellowships are awarded for one year and carry a stipend of $4,020 and an allowance of $600 for travel to the place of the fellowship in Great Britain. Application forms may be secured and submitted to the Executive Secretary, Committee on Growth, Division of Medical Sciences, National Research Council, 2101 Constitution Avenue, Washington 25, D. C.

Excerpta Medica.—*Excerpta Medica,* a monthly abstracting service drawing on articles in every available medical journal in the world, was started in 1947 by the government of the Netherlands. It is now organized on a "not for profit" basis. The board of chief editors consists of M. W. Woerdeman, chairman; Morris Fishbein and W. P. C. Zeeman.

As soon as the *Quarterly Cumulative Index Medicus,* published by the American Medical Association, is brought up to date, its editors hope to indicate in the index references to abstracts that will be easily available in *Excerpta Medica. Excerpta Medica* is published in fifteen sections, each of which may be subscribed to separately. Section 5 is on general pathology and pathologic anatomy.

Books Received

HISTOLOGIE UND MIKROSKOPISCHE ANATOMIE DES MENSCHEN: I. ZELLEN- UND GEWEBELEHRE. By Wolfgang Bargmann, M.D., professor of anatomy, University of Kiel, Germany. Pp. 210, with 192 text figures and 1 colored plate. Stuttgart, Germany: Georg Thieme, 1948.

On the surface this excellent first volume of a textbook of histology does not reveal the difficulties which beset scientists, writers and publishers in present day Germany, for the quality of its paper and printing and the clarity of the illustrations (including photomicrographs) measure up to the traditional high merit of German book making. But because of the loss, during the war, of the author's collection of preparations and figures, only the cooperation of his colleagues at other universities, and finally the energy of the publisher, Dr. Bruno Hauff, made possible the appearance of the book.

Dr. Bargmann, a pupil of von Möllendorff, has succeeded in his endeavor to present to the student a more modern approach to the study of microscopic anatomy; that is, instead of restricting himself to the usual descriptions of static form, he gives a conception of the dynamic changes of the microscopically visible structures on the basis of their submicroscopic construction.

The first part of the book, dealing with cytology—in the broader sense, with living matter, since it includes intercellular substance—occupies 65 pages. In this short space the author has done an admirable piece of writing. He introduces the beginner clearly to the different methods of studying living as well as fixed and prepared material. He brings the information up to date, mentioning (1) histochemical and quantitative methods, (2) ultraviolet, polarization, fluorescence, roentgen, phase and electron microscopy and (3) the use of supersonic waves to divide matter into smallest fragments and so permit investigation to advance into the molecular dimensions of the realm of form. Under the caption "The Cell" he takes up: (1) the structure of the nucleus and its parts; (2) the cytoplasm, its organoids, its paraplasma (cell inclusions—granules, vacuoles, crystals, etc.—of lipids and lipochromes, glycogen, proteins, melanins, etc.), its metaplasma, the formative basis of specific functions (neurofibrils, myofibrils, tonofibrils, etc.) and its hyaloplasma, or fluid substrate; (3) ultrastructure of the living mass, 8 pages being given to its discussion; (4) cell growth and multiplication (mitosis, meiosis and amitosis); (5) functional morphology of cells (mechanics, kinetics, metabolism, irritability); (6) aging and death of the cell. Throughout the book one never loses sight of the bridge between morphology and structural chemistry and physiology.

The second part of the book presents the epithelial tissues, the connective and supporting tissues, the muscular tissues and the nervous tissues. Any classification is, of course, arbitrary, and that of the fundamental tissues becomes a bed of Procrustes, irrespective of which point of view—genetic, structural or functional—one chooses as a basis of systematization. But that Bargmann, to escape part of the dilemma, should relegate the discussion of blood and lymph to the chapter on the circulatory system (to appear in volume 2, on microscopic anatomy or organology) is like cleaving through the Gordian knot, if the reader will excuse another simile here. He follows the classification of Kölliker (Handbuch der

Gewebelehre des Menschen, Leipzig, W. Engelmann, 1855), who listed the four groups of tissues just indicated. Studnička (Allgemeine mikroskopische Anatomie und Organization der lebendigen Masse, in von Möllendorff, Wilhelm, editor: Handbuch der mikroskopischen Anatomie des Menschen, Vienna, Julius Springer, 1929, vol. 1, pt. 1) placed blood and lymph in a fifth group, a classification with which most histologists agree. But Maximow (in von Möllendorff's Handbuch, 1927, vol. 2, pt. 1) classified the blood forming and destroying tissues with the connective tissues, and Bloom (in Maximow, A. A., and Bloom, W.: Textbook of Histology, ed. 5, Philadelphia, W. B. Saunders Company, 1948) put the fully formed blood and lymph there. This solution seems even more far fetched than Bargmann's. Despite the fact that in morphology categoric lines cannot be drawn perfectly, the functional distinctions between blood and lymph and their cells and the connective tissues are sufficiently definite to warrant separate grouping. (Incidentally, the reviewer has always wondered why histologists have failed to recognize germinal, or better procreative, that is, spermiogenic and oogenic, tissues as a sixth—really the first—group of fundamental tissues.)

Bargmann's book is to be recommended to medical students. Even investigators who are working with histologic methods in physiology, pharmacology, surgery and other fields will find it stimulating. Its style is lucid, concise and orderly, and many of the numerous illustrations are original. A combined name and subject index facilitates its use as a textbook.

TUMORS OF BONE. Charles F. Geschickter, M.D., professor of pathology, Georgetown University Medical School; consultant in pathology, United States Naval Medical School; consultant in pathology, Mt. Alto Veterans Administration Hospital; pathologist-in-chief, Gallinger Municipal Hospital, Washington, D. C.; and Murray M. Copeland, M.D., professor of oncology, Georgetown University Medical School; consultant in surgery, Gallinger Municipal Hospital, Washington, D. C.; special consultant, Federal Security Agency, Public Health Service, Cancer Control Branch, Washington, D. C. Third edition. Pp. 810, with 642 illustrations. Price, $17.50. Philadelphia, London and Montreal: J. B. Lippincott Company, 1949.

This book is the product of a wide survey of the literature of bone tumors and allied conditions and a study of a large clinical material, much of which has been followed over long periods. Special emphasis has been given to gross and microscopic pathologic investigation and its correlation with roentgenologic and other diagnostic procedures in the care of the patient. Long term follow-up studies have been made of the results of various forms of treatment.

In view of the general excellence of these sections it is a question whether some other sections, such as the introductory one on interpretation of clinical findings and endocrinopathies and rare diseases of bone, might better have been deleted and space allowed to a more lengthy and detailed discussion of the surgery of bone tumors. Under cancer, more might have been said about the level of amputation and the removal of regional lymph nodes. There is no discussion of extensive resection and bone transplantation for selected cases of bone sarcoma. Nonoperative therapy, including the use of roentgen radiation, radium, isotopes, androgen and estrogen, nitrogen mustards and urethane, are concisely discussed, with the results to be expected from their use in different types of tumors.

In any classification of bone tumors defects will inevitably persist until more is known about the etiologic factors. In general, the gross and microscopic

descriptions of the tumors reported, both cancerous and noncancerous are of a high order, but exception may be taken to the nomenclature employed for some of them. The noncancerous tumors are well classified according to tissue or cell type, but the same procedure is not followed for the cancerous tumors. Instead, their classifications follow Ewing in large measure, who stated that all cases of sarcoma beginning in bone, whether or not the sarcoma comes from bone-forming cells, should be classified as cases of osteogenic sarcoma, but who did not adhere strictly to the rule himself. Thus cancers derived from bone-forming cells are designated as osteogenic sarcoma—osteoblastic or sclerosing type. But cancerous cartilaginous tumors are of three types, osteogenic sarcoma—primary chondrosarcoma, osteogenic sarcoma—secondary chondrosarcoma, and chondroblastic sarcoma.

Tumors generally designated as angiosarcoma or malignant bone aneurysm have been classified as "osteolytic osteogenic sarcoma" because the observers considered them to consist of cancerous forms of plump spindle cells and pleomorphic osteoblasts. An attempt is also made to place some tumors rich in giant cells in this class. These are ill advised changes, as the term gives no clue to the histologic aspect of the tumor and in all instances sarcoma is more or less osteolytic or bone absorbing. Fibrosarcoma, which usually produces extensive central erosion of bone, is considered always to be of periosteal origin, a finding not substantiated by the great majority of experienced pathologists. Ewing's sarcoma or endothelial myeloma begins in the bone, but despite this fact, it is not classified as a type of osteogenic sarcoma. Neither is it considered an endothelioma, as Ewing held. Ewing's tumor is thought to originate most likely from the reticulum cells lining the sinuses of lymphoid tissue and giving rise to cells of the lymphocytic series. There is no discussion of reticulum cell sarcoma as described by Parker and Jackson, but reference is made to the fact that bone metastases of neuroblastoma are frequently diagnosed incorrectly as Ewing's sarcoma. In order to avoid the confusion created by this nomenclature, it would be better to drop the term "osteolytic osteogenic sarcoma," as well as "Ewing's tumor," which is now recognized as designating not an entity but various round cell sarcomas; also to limit "osteogenic sarcoma" to the cancers arising from cells of osteoblastic origin, and to classify sarcomas, as well as benign tumors, of bone according to tissue or cell type.

A worth while contribution has been made in recognizing periosteal osteoma as an entity. It has usually been mistaken for sarcoma and wrongly treated by amputation instead of by wide excision.

There are many valuable sections on tumors of regions of the skeleton and on allied conditions which must be differentiated from bone tumors.

The book will prove useful to pathologists, orthopedic and general surgeons and internists.

STUDIES IN AIR HYGIENE. By R. B. Bourdillon, O. M. Lidwell and J. E. Lovelock, with W. C. Cawston, L. Colebrook, F. P. Ellis, M. Van Den Ende, R. E. Glover, A. M. MacFarlan, A. A. Miles, W. F. Raymond, E. Schuster and J. C. Thomas. Medical Research Council Special Report Series no. 262. Pp. 356. Price 7s 6d. London, England: His Majesty's Stationery Office, 1948.

The fear that World War II might be associated with great epidemics of influenza and other "air-borne" infections led to the organization, in London, in 1940, of a team of investigators under the direction of the late Sir Patrick Laidlaw to explore new means of air hygiene. After Laidlaw's death the direction of the work fell to Dr. C. H. Andrewes and later to Dr. R. B. Bourdillon. Report 262.

which the Medical Research Council justifiably calls "a landmark in the study of air hygiene," is a collection of forty-four papers by members of the group, plus an appendix dealing with certain technical aspects of the work.

The first twelve papers deal with the problem of air sampling. The ingenious and useful "slit sampler" invented by Dr. Bourdillon provides the background for most of these papers. Theoretic and practical problems presented and solved by the slit sampler are discussed, and the various "models" of the sampler are described. Though the slit sampler has not been employed in work on air-borne infection in the United States, there is no doubt that it is one of the most important tools so far developed in this field. Its particular merit is that it permits the bacteriologic content of the air to be measured continuously and accurately before, during and after the use of bactericidal agents.

"Air Disinfection by Chemicals" is the title of the second section of the report. Herein are described the experiments carried on in the search for a chemical agent which would fulfil the rigid requirements of a "perfect" air sterilizing agent. Though the ideal compound has not yet been found, much progress has been made. The British and American workers are in agreement that the action of the chemicals advanced to date, which are effective against air-borne micro-organisms in concentrations of micrograms or even fractions of a microgram per liter of air, occurs when the chemical in vapor phase condenses on the air-borne particle. Thus, it is incorrect to speak of these chemicals as "aerosols." In the United States, propylene and triethylene glycol have been the subjects of most of the investigations of bactericidal vapors. Bourdillon and his group explored the properties of a long list of compounds, of which lactic acid and alpha-hydroxy-alpha-methyl butyric acid are the most promising. Both appear to be active against air-borne bacteria carried in dry particles, a property not shared by many "air-sterilizing" agents.

Studies with ultraviolet rays attracted some attention. The limitation placed on the usefulness of this method by the fact that the radiation has to be restricted to the upper third of a room because of danger to the eyes is well recognized. Other methods of air disinfection studied included the use of heat (especially for the air of laboratories dealing with dangerous pathogens), the passage of air through filters and the use of masks.

Dr. Bourdillon and his group appreciate the great difficulties which lie in the way of determining just how many infections of the respiratory tract are strictly air borne. However, they rightly point out that there is much justification for attempting to reduce the load of air-borne micro-organisms by the methods now at our disposal. Though no data concerning the infecting dose of respiratory pathogens for human beings are available, animal experiments have shown that both the severity and the mortality of air-borne infections are directly influenced by the dosage of virus or bacteria. They discuss also the argument that reduction of the concentration of air-borne bacteria is a bad thing because it will ultimately impair human immunity to these pathogens. They do not hold with this argument and point to the perhaps debatable analogy with water hygiene to support their contention. This reviewer is in accord with their general conclusions. Though in the case of such infections as measles and chickenpox it is advisable for children to acquire immunity by having the clinical disease before they are fully grown, there is no evidence that this principle applies to common respiratory pathogens. Furthermore, no means of air disinfection is likely ever to accomplish 100 per cent reduction of the number of air-borne micro-organisms in all places where human beings meet.

TEXTBOOK OF HISTOLOGY. By Jose F. Nonidez, D.Sc., late professor of anatomy, Cornell University, and professor of microscopic anatomy, University of Georgia, and William F. Windle, Ph.D., Sc.D., professor of anatomy, University of Pennsylvania. Pp. 456, with 287 illustrations (209 drawings and diagrams, and 193 photomicrographs). Price $6.75. New York: McGraw-Hill Book Company, Inc., 1949.

Professor Nonidez had been working for some time on the illustrations and manuscript of a textbook of histology when he died in the autumn of 1947, only a few weeks after arriving at the University of Georgia. Some weeks later Professor Windle took on the task of completing the work as nearly as possible according to the plan laid down by Nonidez, who had stated his objective in the following way: "The purpose of the proposed book is to present in concise form the fundamental facts on the finer structure of the mammalian body, including man, and to emphasize as far as possible the functional aspects. . . . It was soon realized that a book with a concise text would have to be adequately illustrated and that figures would fit the text better if they were drawn for the purpose instead of being borrowed from other textbooks and scientific journals. . . . In preparing the text, it has been borne in mind that the inclusion of controversial subjects, names of authors and references make reading difficult. . . . Many students lack proper preparation for the study of histology. The situation has been taken into account, and little knowledge of anatomy and physiology is taken for granted."

Accordingly, the textbook is meant for the beginner. It is not a reference work, for not only are many details lacking but also a comprehensive bibliography. In the appendix Windle has listed 18 histologic, embryologic and physiologic textbooks in English which he used in the preparation of this book and to which he directs the exceptional student for bibliographic information. Too, at the end of each chapter, he gives two or three selected references, which he annotates pertinently in order to guide the interested student to such collateral reading.

Since only one half of the text existed in rough copy at the time of Nonidez's death, Windle found it expedient, for the sake of coherence of description and consistency of style, to rewrite even that part. His method of approach is adapted to the novice. The sentences are short, simple and direct; indeed, frequently they begin with the pronoun "you" or imply it. There is little adornment of phraseology, and only exceptionally is the dramatic element exploited.

The great majority of the 200 drawings and diagrams were made by Nonidez. For the most part they are original, are executed simply and express clearly the points to be illustrated. The equal number of remaining figures are photomicrographs, most of which were taken under Dr. Windle's direction. Many of these are excellent, especially those that were taken under the lower magnifications to serve as orientation pictures in the study of histologic relations and construction. On the other hand, there is a relatively large number—30 or 40—of other photomicrographs, taken under higher magnifications, which are unsatisfactory because of poor focus and lack of definition and contrast. These are more confusing than helpful to the beginner. Doubtlessly, better ones will take their place in a second edition. Windle is to be commended for stating in every case the magnification employed in the preparation of the figure.

Windle is in accord with all alert teachers of cytology and histology regarding the desirability of making the subject come alive. Few histologic laboratories at present are equipped to set up in vitro or in vivo experiments for the benefit of the beginning student. Hence, increasingly greater use is made of motion pictures for conveying to him the concepts of living cells and tissues. To assist the student

and the teacher in the selection of suitable motion pictures to supplement the lectures and the laboratory work, Windle has appended to his textbook a reference list of films and their sources.

The Nonidez and Windle textbook represents a fine piece of book-making by the publishers, McGraw-Hill. The appearance is pleasing, and the binding, the quality of paper and the printing are excellent. On the whole, it is well edited, and it is notably free of typographic errors. The reviewer believes it will become a popular book with beginning students of histology.

ATLAS OF PERIPHERAL NERVE INJURIES. By William R. Lyons, Ph.D., associate professor of anatomy, University of California Medical School, and Barnes Woodhall, M.D., professor of neurosurgery, Duke Medical School, Durham, N. C. Price $16. Philadelphia: W. B. Saunders Company, 1949.

This handsome volume presents an enormous amount of material, much of it of great interest to pathologists, as well as to anatomists and neurosurgeons. In the foreword, Dr. Glen Spurling comments that the book, a product of World War II, "differs from many other research projects accomplished during the war in that it was supported by no elaborate budget and was achieved by the authors while they were carrying, at the same time, a heavy load of clinical and administrative responsibilities."

The book embodies the experience accumulated by the authors in dealing with peripheral nerve injuries in the Walter Reed and Halloran General Hospitals during 1943 to 1945, supplemented by specimens contributed by other neurosurgeons. In the course of the day to day work some 3,000 photographs and photomicrographs were collected. There are thus presented several hundred photographs, with accompanying text, all portraying peripheral nerve injuries, and including clinical illustrations as well as those of gross and microscopic pathology.

After a brief discussion of terminology and of peripheral nerve structure, as well as of methods of staining and fixation, the remainder of the volume is divided into four sections, dealing respectively with completely severed nerves, traumatic nerve lesions without loss of continuity, nerve sutures and nerve grafts. In each section there is a relatively brief introduction, followed by many dozen fine photographs and photomicrographs. There are 135 pages of plates, each with a facing page of descriptive legends, so that illustrations and legends together occupy 270 of the book's 331 pages.

A question may well be raised regarding the wisdom of the mode of presentation. The texts are relatively brief and do not furnish any comprehensive analysis of the subjects treated, although 195 bibliographic references are made. The photographs are excellent, but there is a certain repetitiveness which should not obtain in an atlas. There are many illustrative cases, with data on the clinical course, and photographs of gross and microscopic specimens. These are of great concrete value and interest, more so, perhaps, than the array of photographs exemplifying general changes. Among the latter, however, there are many fine illustrations which will be greatly appreciated by all pathologists. Many of the plates are in color, adding materially to the cost of the book without compensatory enhancement of its scientific worth.

The sections on nerve suture and nerve grafts are of especial interest, but the texts are far too brief. It appears to this reviewer that if, in the entire book, the illustrations were reduced to a quarter of their number and the text expanded tenfold, the volume would be of greater value. But then, perhaps, it would no longer be an atlas.

PATHOLOGISCHE PHYSIOLOGIE DER FRISCHEN, GESCHLOSSENEN HIRNVERLETZUNG, INSBESONDERE DER HIRNERSCHÜTTERUNG; KLINISCHE, ANATOMISCHE UND EXPERIMENTELLE BEFUNDE. NEBST ANHANG: THERAPEUTISCHE FOLGERUNGEN. By R. Wanke, professor of surgery at the University of Kiel. Pp. 196, with 90 illustrations. Stuttgart: Georg Thieme Verlag, 1948.

The first section of this monograph deals principally with experimental and clinical observations made in cases of acute cerebral concussion. The number of animal experiments is limited, and the experimental work is not thorough. The author has attempted to prove that many of the phenomena of cerebral concussion may be referred to a disturbance of the autonomic nervous system due to involvement of autonomic centers of the midbrain and neural pathways connected with these centers. The evidence offered in proof of this thesis is not convincing.

A second section of the monograph is concerned with cerebral edema, intracerebral hemorrhage and changes of cerebrospinal fluid pressure occurring in patients with acute cerebral contusion or concussion. The observations here are generally in accord with those which have been well recognized for some time.

The final section of the monograph deals with the treatment of the patients and the prognosis. Except for necessary measures in handling complications, the conclusion is reached that the best method of treatment is that of noninterference.

This volume adds little to the existing body of information about cerebral concussion, its complications and method of treatment. In some respects, the data add further confusion, because this author, as well as most others, has failed to recognize that a critical approach to an analysis of the causes of the disturbances following acute cerebral injury and a proper evaluation of treatment should be conducted by methods which are capable of reproducing, qualitatively and quantitatively, acute cerebral injury, either of local or of generalized distribution.

THE 1948 YEAR BOOK OF PATHOLOGY AND CLINICAL PATHOLOGY. Edited by Howard T. Karsner, M.D., professor of pathology and director of the Institute of Pathology, Western Reserve University, Cleveland, assisted by Herbert Z. Lund, M.D., assistant professor of pathology, Western Reserve University, Cleveland. Clinical Pathology: Edited by Arthur Hawley Sanford, M.D., professor of clinical pathology, University of Minnesota (Mayo Foundation), and senior consultant, Division of Clinical Laboratories, Mayo Clinic. Pp. 538, with 127 illustrations. Price $4.50. Chicago: The Year Book Publishers, 1949.

This addition to the series of Year Books presents an adequate survey of the fields of pathology and clinical pathology and will be valuable not only to pathologists but to internists, surgeons and others as well. A notable feature, included in the section on pathology, is a short review of articles which give a good synthesis of recent work on shock, cytologic diagnosis of tumors, vitamin B_{12}, pulmonary berylliosis and iron metabolism. These articles are most helpful and could be increased in future editions. The articles selected for inclusion are well chosen and the abstracts are of sufficient length to do justice to the original. The contents of the book are too numerous to be mentioned in this review, and it is hoped that this will induce others to read it for themselves. They will be richly rewarded.

HISTOLOGIA DE LARINGE, TRAQUEA, BRONQUIOS Y PULMONES. By Manuel Perea Muñoz, profesor adjunto de anatomía patológica y jefe del laboratorio de anatomía patológica del Instituto de Tisiología de la Facultad de Ciencias Medicas de Cordoba. Pp. 59, with 12 illustrations. Buenos Aires, Argentina: Ediciones Aguamarina, 1948.

ÜBER NEUROME UND NEUROFIBROMATOSE, NACH UNTERSUCHUNGEN AM MENSCHLICHEN MAGENDARMSCHLAUCH. By F. Feyrter, O. professor der Pathologie, Georg Hanusch-Krankenhaus der Wiener Gebietskrankenkasse für Arbeiter und Angestellte, Wien. XLV. Pp. 125, with 37 illustrations and 6 tables. Price, $3.50. Vienna, Austria: Verlag Wilhelm Maudrich (New York: Grune & Stratton, Inc.) 1948.

The book is divided into four sections and deals with neuroma, Recklinghausen's neurofibromatosis and fibroma of the gastrointestinal tract. In section 1, the types of neuroma are given as follows: (*a*) the fusiform, (*b*) the multiform, (*c*) the microcytic, (*d*) the reticular, (*e*) the granular, (*f*) the myoneuroma and (*g*) the macrocytic type. They are distinguished from the myoma. They arise from or in the region of the Meissner and Auerbach plexuses. The sites and the frequency of occurrence are discussed. Frequently neuroma occurs in the stomach and the small intestine. The fusiform type is the most common, being found in three fourths of all cases.

Section 2 discusses Recklinghausen's neurofibromatosis of the gastrointestinal tract. These tumors are derived from the endoperineural tissue and appear with and without cutaneous neurofibroma. They are found in the small intestine, the large intestine and the rectum and are polypoid or plate-shaped. They are composed of ganglion cells, satellite cells, Schwann's cells and endoperineural cells. Some are associated with capillary hemangioma.

Fibroma of the gastrointestinal tract is discussed in section 3. It invariably occurs in patients older than 45 years of age. It is found in the ileum in submucous position and is more frequent in men than in women. It arises from the perineural tissue.

In section 4 certain glandular polyps of the gastric pylorus are discussed, which have an interstitial origin from endoperineural covering and nerve network. Growths of this sort are restricted to that portion of the mucous membrane in which the neurogenous Meissner plexus is developed. One type is composed of acellular fine fibers without many capillaries, and the second type is composed of large fibers and has a vascular appearance.

The book expresses the author's views based on his experiences.

A YEAR WITH OSLER 1896-1897: NOTES TAKEN AT HIS CLINICS IN THE JOHNS HOPKINS HOSPITAL. By Joseph H. Pratt, a Member of the Class of 1898. Cloth. Price, $4. Pp. 209, with 6 illustrations. Baltimore: Johns Hopkins Press, 1949.

Osler and his clinical work in 1896-1897 are well described in the introduction and preface. The photograph of Osler at that time and the five plates are good and highly interesting. The "clinical notes" (198 pages) reproduce accurately clinical talks and expositions by Osler. The book is a valuable record "not only of a great teacher, but of great teaching."

CLINICAL CHEMISTRY IN PRACTICAL MEDICINE. By C. P. Stewart, M.Sc. (Dunelm.), Ph.D. (Edin.), Reader in Clinical Chemistry, University of Edinburgh; Senior Biochemist, Royal Infirmary, Edinburgh; and D. M. Dunlop, B.A. (Oxon.), M.D., F.R.C.P. (Edin.), F.R.C.P. (Lond.), Christison Professor of Therapeutic and Clinical Medicine, University of Edinburgh; Physician, Royal Infirmary, Edinburgh. Third edition. Price $5. Pp. 324, with 30 illustrations. Baltimore: The Williams and Wilkins Company, 1949.

One of the purposes of this book is to give information on the circumstances in which a chemical examination may be of service in diagnosis and prognosis of

diseases and in control of therapy. A second purpose is to give an appreciation of the rationale of chemical pathology in its application to practical medicine, though what is meant by "practical" is not made clear. A third purpose is to provide a broad knowledge of how various chemical analyses are carried out and a detailed knowledge of the method of performing many of the simpler tests which do not require much time or equipment and which may be performed in the physician's own surgery or dispensary. The book is addressed to the practitioner, house physician and senior student.

Most of the material in the book will be familiar to all physicians. The group to whom the book is addressed will not be stimulated by its contents, for most of the methods are too complicated and their interpretations too devious. Of the simple tests described, those which may be carried out in a physician's office by the physician or nurse are tests for sugar and protein in the blood, urine and spinal fluid, lactic acid and hydrochloric acid in the gastric juice, the amylase tests for acute pancreatitis, the van den Bergh and the sulfobromophthalein sodium tests for hepatic function and the erythrocyte sedimentation test. Not more than twenty pages would be needed to deal adequately with those tests. The boredom with which a practitioner will read this book is due to its failure to give the rationale of chemical pathology which it sets out to do. Insofar as the book reflects the desires of the chemist, it gives good advice on the collection and preservation of samples for analysis.

STUDIES ON HOOKWORM DISEASE IN SZECHWAN PROVINCE, WEST CHINA. By K. Chang and co-workers. American Journal of Hygiene Monographic Series, no. 19, May 1949. Supported by the De Lamar Fund of the Johns Hopkins University. Pp. 152, illustrated. Price $3. Baltimore: The Johns Hopkins Press, 1949.

Using the smear technic, the Willis brine flotation technic and the Stoll dilution egg-counting method in the examination of fecal specimens, the authors obtained accurate information on the severity of hookworm disease in the separate areas of the province, the population of which is estimated at 45,000,000. Their findings were then broken down into occupational and age groupings and correlated with existing climatic and agricultural conditions. The conclusions reached point the way toward a clearer understanding of the many factors involved in the treatment and control of hookworm disease. There are numerous graphs and charts which further interpret this exacting biologic study.

CANCER OF THE ESOPHAGUS AND GASTRIC CARDIA. Edited by George T. Pack, B.S., M.D., clinical professor of surgery, New York Medical College; attending surgeon, Memorial Hospital for the Treatment of Cancer and Allied Diseases, New York. Pp. 192, with 25 illustrations. Price $5. St. Louis: V. C. Mosby Company, 1949.

This is a reprint of twelve articles on the present treatment of cancer of the esophagus and gastric cardia in the June 1948 number of *Surgery*.

KOSMETIK UND ALLGEMEINE PATHOLOGIE. By Dr. Med. Franz Halla. Pp. 118. Price $2.50. Vienna, Austria: Verlag Wilhelm Maudrich (imported by Grune & Stratton, New York), 1948.

This booklet deals with the relations between the skin and systemic disease, which the author claims have been neglected in the literature. It does not, however, go into the problems thoroughly.

INDEX TO VOLUME 48

Subject entries are made for all articles. Author entries are made for original articles. Book Reviews and Obituaries are indexed under these headings in alphabetical order under the letters B and O, respectively.

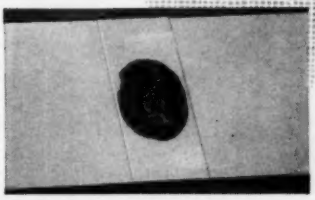
This Slide Method gives (Rh) test in seconds

1 • *Place two drops of blood on slide. Fresh, clotted, oxalated, heparinized or citrated blood may be used.*

2 • *Add one drop of Anti-***Rh** *serum.*

3 • *Mix Anti-***Rh** *serum with blood and spread over slide.*

4 • *Place slide on view box. Tilt view box back and forth and watch for clumping. No microscope is needed.*



5 • *Clumping starts in 45 seconds, is complete in 1 to 2 minutes. Clumping indicates blood is* **Rh** *Positive; absence of clumping,* **Rh** *Negative.*

The Slide Method, using the view box, accounts in part for the rapidity and accuracy of **Rh** Tests. But even more important is the potency of the serums themselves.

Prepared under the strictest controls, and according to N. I. H. specifications, AMERICAN serums retain their original potency over a long period even under adverse conditions. Six strategically located distributing centers assure you of prompt service. Write today for complete descriptive literature.

PLAN WITH AMERICAN
...the first name in hospital supplies

AMERICAN HOSPITAL SUPPLY CORPORATION

GENERAL OFFICES • EVANSTON, ILLINOIS

While Patients Wait....

LET THEM READ HYGEIA

Offer them the opportunity to gain a new viewpoint on medical science and a finer appreciation of your services.

HYGEIA tells the story of medical service in a wholesome, common sense manner, true to the spirit of scientific medicine—yet in thoroughly readable style and attractive format.

Helping to lay firmer foundations of patient cooperation, combating the flow of inaccurate health information from unreliable sources, exposing quackery and "sure cures," telling the fascinating story of medical progress in lay language—HYGEIA can work silently, side by side with you, day in, day out.

Make sure there is a copy of HYGEIA in your waiting room *every month*. Use the coupon below to order—TODAY!

Gentlemen: Start sending HYGEIA to the address below at once. ☐ Bill me next month (OR) ☐ I enclose $3.00 for one year's subscription (OR) ☐ I enclose $5.00 for two years' subscription.

DR.....

ADDRESS.....

AMERICAN MEDICAL ASSOCIATION, 535 N. Dearborn St., Chicago 10, Ill.